PS01 CD4+ Regulatory T Cells Prevent Helicobacter hepaticus-Induced Colon Cancer in Rag2-Deficient Mice

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Intestinal microbiota and host immunity are integrally linked in large bowel disease and health. Recent studies show that the adaptive immune arm is particularly important in protecting against primary tumor development of intestinal epithelia, but the functions of lymphocytes in this location have not been characterized. To better understand the relationships between intestinal bacteria, immune cells and carcinoma, 129/SvEv Rag2-deficient and 129/SvEv wild type mice were orally inoculated with a widespread enteric mouse bacteria, Helicobacter hepaticus (n = 80), or sham-dosed with media only (n = 30). Inflammatorily, hyperplastic, and neoplastic bowel lesions were quantified on a scale of 0-4 with ascending severity, and then compared using Mann-Whitney U nonparametric test for categorical data. Helicobacter-infected Rag2-/-, but not sham-dosed Rag2-/- mice, rapidly developed colitis (P = 0.0001) and large bowel carcinoma (P = 0.0001), demonstrating a link between microbially-driven inflammation and cancer in the lower bowel. Helicobacter-infected wild type mice did not develop inflammation or carcinoma, indicating that adaptive immunity was required to prevent microbially-induced cancer at this site. Adoptive transfer with CD4+, CD4+ CD25+, or more highly purified CD4+CD25+CD45RBlo regulatory T cells prior to infection with Helicobacter significantly suppressed inflammation (P < 0.00001) and prevented development of cancer (P < 0.00001). These results suggested that CD4+ regulatory T cells protected against cancer in the intestinal epithelia primarily by preventing bacterially induced innate immune dysregulation at this site. These findings propose a novel role for T cells in protection against colon carcinoma and have implications for new modes of prevention and treatment of cancer in humans.

PS02 Concomitant Infection with Both Helicobacter bilis and Helicobacter hepaticus Modulates Colitis Phenotype and May Promote Tumorogenesis in Multiple Drug Resistance Deficient (mdr1a/-) Mice

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Mdr1a-/- mice are deficient in the membrane transporter P-glycoprotein and develop spontaneous colitis with age. Infection with H. bilis can accelerate colitis, while infection with H. hepaticus infection delays development of colitis in these genetically susceptible mice. We sought to determine if co-infection of mdr1a-/- mice with H. hepaticus would modulate or prevent the development of H. bilis-induced colitis. Three- to 5-week-old female mdr1a-/- mice were orally infected with H. bilis alone (n = 10), H. bilis followed by H. hepaticus (n = 20), H. hepaticus followed by H. bilis (n = 20), H. hepaticus alone (n = 20) or broth (n = 20). Mice were housed under SPF conditions and monitored for weight loss or diarrhea and, following euthanasia, necropsied to evaluate colitis histopathologically. By 16 weeks postinfection (PI), colitis incidence differed among the groups: 100% in H. bilis alone, 53-65% in both H. bilis and H. hepaticus co-infected groups, 5% in H. hepaticus alone, and 60% in broth (spontaneous colitis). Hence, at 16 weeks PI, the presence of H. hepaticus significantly altered the incidence of H. bilis-induced colitis (P = 0.03). However, by 28 weeks PI, colitis incidence was the same in all groups (85-100%) except H. hepaticus alone (25%). Most animals showed typical colitis lesions including crypt hyperplasia and branching, crypt abscesses, and obliteration of normal gut architecture. Unexpectedly, a proportion of mdr1a-/- mice co-infected with both H. bilis and H. hepaticus exhibited dysplastic changes with polypoid lesions in colonic and rectal tissue that were consistent with carcinoma in situ. Immunohistochemistry is currently being done to assess expression of Cox-2, CD44 and beta-catenin to further phenotype these lesions. Our findings suggest that the presence of differing enteric Helicobacter spp. can alter the incidence and phenotype of colitis and may promote colon tumorogenesis in a host with deficient intestinal P-glycoprotein.

PS03 Cytotolethal Distending Toxin Is a Newly Identified Putaive Virulence Factor in Helicobacter cinaedi

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Helicobacter cinaedi has a wide host range that includes hamsters, dogs, humans, and most recently was reported in a colony
of rhesus macaques with idiopathic colitis. Interestingly, *H. cinaedi*, a suspected zoonotic bacterium, is the most commonly reported enterohelicopter helicobacter in humans; it causes a variety of diseases, particularly in immunocompromised hosts, including gastritis, proctitis, bacteremia, cellulitis, and arthritis. While it is an important pathogen, virulence factors have not been reported in *H. cinaedi* and little is known about its pathogenesis. Recently, other enterohelicopter helicobacters have been reported to produce a toxin belonging to the family of proteins associated with cell division at the G2/M phase (range = 32-80% of cells arrested), cal morphological changes in tissue cultured cells, arrested cell division in the G2/M phase (range = 32-80% of cells arrested), and had the expected 700 bp PCR product. Surprisingly, the *H. cinaedi* lacked both the gene and activity in cell culture. Sequence of the 16S rRNA gene of the ATCC type strain indicated that it was *H. fennelliae* and not *H. cinaedi*. Cloning and sequencing of the gel purified 700bp PCR product from two *H. cinaedi* isolates showed 96% homology with the cdhB of helicobacter 96-6070 (AF243080), 68% homology with helicobacter 98-6070 (AF243079) and *H. hepaticus* (AF163666) cdhB, and 56% homology with *H. bilis* cdhB (AF243077). While the role of this toxin in disease is unknown, *H. cinaedi* shares the production of this toxin with other enteric pathogens including pathogenic *Escherichia coli*, *Campylobacter jejuni* and *Shigella* spp. To our knowledge, this is the first report of a putative virulence factor in *H. cinaedi*.

PS04 Experimental *Helicobacter marmotae* Infection in A/J Mice Causes Enterohelicopter Disease

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Recently *Helicobacter marmotae* sp. nov. was recovered from the inflamed livers of Eastern woodchucks (*Marmota monax*) infected with woodchuck hepatitis virus (WHV). The same bacterium has been cultured from WHV-negative woodchucks with hepatitis and the feces of commercially raised cats. Because the majority of woodchucks with hepatic tumors, with or without WHV, were either culture- or PCR-positive for this novel helicobacter, a role for *H. marmotae* in tumor promotion has been proposed. In this study, the bacterium was inoculated into 48 male and female A/J mice, a strain noted to be susceptible to *H. hepaticus*-induced liver tumors. Sixteen mice served as sham-inoculated controls. At 6, 12, and 18 months postinoculation (PI), there were statistically significant (*P < 0.05*) differences in mean inflammation scores for the cecum and proximal colon between experimental and control mice. In addition, typhlocolitis scores were positively correlated with cellular culture results in the experimental mice. Differences in hepatic inflammation were significant (*P < 0.05*) at 6 and 12 months PI between the control and experimental groups, but not at the final 18-month timepoint. Two infected male mice had livers with severe hepatitis that were also culture positive for *H. marmotae*. These results demonstrate that the woodchuck helicobacter can successfully colonize mice and cause gastrointestinal disease. Future work with *H. marmotae* in mice should be useful for dissecting mechanisms of co-carcinogenesis involved in tumor development in woodchucks with WHV, and by extension in humans infected with hepatitis B virus.

PS05 Gastric Adenocarcinoma in *Helicobacter pylori*-Infected Transgenic INS-GAS Mice

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Constitutive gastrin production in mice carrying the insulin-gastrin (INS-GAS) transgene exacerbates gastric disease in helicobacter-infected animals. A high-salt diet is also associated with more pronounced gastric lesions in *Helicobacter pyloril-infected C57BL/6* mice. We asked whether a high-salt diet might accelerate gastric disease in hypergastrinemic INS-GAS mice infected with *H. pylori* Sydney strain 1. Eighty-six INS-GAS mice were divided into equal groups that received *H. pylori* or vehicle only, and were further subdivided to receive either a high-salt (7.5% NaCl) or basal (0.25% NaCl) diet. Histopathology and quantitative *H. pylori* culture of the stomach were performed on mice euthanized 5 and 7 months postinfection. Antral and fundic bacterial colonization increased with time (both *P < 0.00001*) but was not influenced by diet (*P > 0.4*) or gender (*P > 0.5*). In contrast, gastric disease was significantly influenced by gender, diet and time postinoculation (all *P < 0.02*). Microscopic lesions in infected animals included fundic chronic active inflammation, glandular atrophy with a greater loss of chief than parietal cells, and intestinal metaplasia of gastric epithelium. Lesions were most severe in the glandular stomach near the junction with the squamous portion (cardia and oral fundus). Glandular dysplasia had progressed to in situ and/or intramucosal gastric adenocarcinoma in five animals. Four of those five were males on the basal diet at the 7-month timepoint. This confluence of factors in the development of carcinoma mirrored the overall pattern of factors that influenced disease severity; i.e., males were more severely affected than females and animals at the later timepoint were more severely affected than those at the early timepoint. Unexpectedly, animals on the basal diet developed more severe lesions than did those on the high salt diet at 7 months postinfection. The mouse model is consistent with human epidemiologic data demonstrating a greater prevalence of gastric adenocarcinoma in men relative to women. This study highlights the importance of using both genders to investigate the pathogenesis of *H. pylori*-related gastric pathology and its associated carcinogenesis. Further studies are required to determine the specific mechanisms by which hypergastrinemia and high salt diet together and separately influence disease outcome in individuals infected with *H. pylori*.
PS06 Helicobacter Infection Interferes with the Development of Pertussis Toxin-Induced EAE in a Mouse Model of Multiple Sclerosis, Myelin Basic Protein TCR Transgenic Mice

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MBP-TCR transgenic mice have T cells specific for myelin basic protein, a component of the central nervous system, and are used as an animal model for multiple sclerosis. These mice develop experimental allergic encephalomyelitis (EAE) after administration of pertussis toxin. Older (> 6 months) MBP TCR mice, naturally infected with Helicobacter bilis and H. hepaticus, occasionally developed mild clinical signs of colitis. After injection with 400 ng of pertussis toxin (i.v., days 0 and 2) to induce EAE, over a 3-week period the majority of these Helicobacter-positive mice developed severe colitis characterized by diarrhea and greater than 20% weight loss, which resulted in euthanasia (17/22). Only 23% (5/22) developed EAE. A second group of mice (n = 6; Helicobacter-positive) were given 800 ng (i.v., days 0 and 2) of different lot of pertussis toxin to rule out lot-to-lot variation. All six mice developed clinical and histological signs consistent with typhlocolitis and none developed EAE. These experiments led to the hypothesis that pertussis toxin potentiates Helicobacter-induced colitis in MBP-TCR transgenic mice and prevented EAE. Following rederivation as Helicobacter spp. negative, 14 mice were injected with pertussis toxin (400 ng as above). No colitis was noted and 85% (12/14, negative, 14 mice were injected with pertussis toxin (400 ng as above)) developed EAE. A cohort of these Helicobacter-negative transgenic mice was infected with H. bilis and H. hepaticus by oral gavage 3X (2 x 107), confirmed positive by fecal PCR, and given pertussis toxin (400 ng, as above). Over a 3-week period, all (5/5) mice developed severe typhlocolitis and no animals developed EAE (P = 0.00004). Thus, Helicobacter spp. infection in MBP-TCR transgenic mice prevents the development of pertussis toxin-induced EAE and induces typhlocolitis.

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PS07 Helicobacter trogontum Causes Severe Typhlocolitis in B6.129 IL-10−/− Mice

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Helicobacter trogontum is a urease-positive helicobacter isolated from the colon of subclinically infected rats and is closely related to H. hepaticus and members of the Helicobacter (Flexispira) rappini taxon by 16s rRNA sequence analysis. H. trogontum colonizes at multiple levels of the gastrointestinal tract in germ-free mice and was associated with mucosal inflammation in the stomach, small intestine, cecum and colon as well as mild hepatitis in selected mice. We infected B6.129P2-Ili10−/− mice with H. trogontum to study its potential to model inflammatory bowel disease (IBD) because IL-10−/− mice infected with other Helicobacter spp. will develop typhlocolitis of varying severity. IL-10−/− mice from a colony naturally infected with H. rodentium, a urease-negative helicobacter, were dosed with 2 X 107 H. trogontum three times by oral gavage (n = 14) or served as controls (n = 10). Mice were necropsied at 2, 4 or 6 weeks postinfection due to progressive evidence of bloody feces, dehydration and weight loss in H. trogontum infected mice. Controls were not clinically affected by H. rodentium and at necropsy had mild enlargement of mesenteric lymph nodes. All H. trogontum-infected mice had gross evidence of cecal-colic thickening, enlarged mesenteric lymph nodes, and most mice were debilitated. Severe typhlocolitis was observed histologically with transmural infiltration of mononuclear inflammatory cells, epithelial erosions and associated distortion of the normal bowel architecture. Th1-associated serum IgG2a responses to H. trogontum by ELISA predominated over Th2-associated IgG1 responses (P < 0.003). These results suggest that H. trogontum infection in IL-10−/− mice promotes typhlocolitis secondary to an enhanced Th1 immune response and thus may mimic some features of Crohn’s disease in humans. This model appears useful to IBD research because of the acute nature of severe enteric disease secondary to helicobacter infection and will be further studied in helicobacter-free IL-10−/− mice to ascertain whether monoinfection with H. trogontum produces a similar disease.

PS08 Interaction of Enterohepatic Helicobacter Species with Macrophages: Receptors Involved in Uptake and Signaling

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Infection of susceptible strains of mice with enteroheligmatic Helicobacter species (EHS) such as H. hepaticus or H. bilis results in chronic-active hepatitis and/or inflammatory bowel disease (IBD). Mice remain persistently infected although EHS elicit a robust Th1-mediated immune response. Proinflammatory cytokines produced by the immune system are believed to play a role in the pathology associated with infection. To better understand how immune responses are initiated following EHS infection, we investigated the interaction of H. hepaticus and H. bilis with mouse macrophages in vitro. The murine macrophage cell line, J774A.1, was cultured in the presence or absence of E. coli O26:B6 LPS for 24 h prior to infection with H. hepaticus or H. bilis. In some instances, bacteria were opsonized with normal mouse serum prior to infection. Phagocytosis was analyzed by immunofluorescent staining using Hoechst dye for the detection of bacteria and phaloidin, a dye specific for F-actin in mammalian cells. IL-12 production was measured by ELISA. Our data demonstrates that LPS-treated J774A.1 macrophages are poorly phagocytic for EHS in the absence of opsonization of bacteria, possibly implicating the complement receptor in bacterial uptake. Unlike H. pylori, H. hepaticus and H. bilis do not appear to survive intracellularly in macrophages suggesting that persistent infection is not due to an inability of macrophages to kill internalized bacteria. Interestingly, culture conditions that are optimal for phagocytosis of H. hepaticus and H. bilis result in minimal production of IL-12. However, preincubation of macrophages with IFNγ results in high levels of IL-12 production following infection with EHS. Our results indicate that distinct receptors are involved in uptake of EHS and production of proinflammatory cytokines by murine macrophages.

PS09 Xeno-Infection of Nonhuman Primates by Feline Immune Deficiency Virus

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New viral infections in humans usually result from viruses that have been transmitted from other species as a zoonoses. It is accepted that human immunodeficiency virus is the result of
propagation and adaptation of a simian immunodeficiency virus (SIV) from nonhuman primates to man. Hypothesis: Feline immunodeficiency virus can be experimentally transmitted to a primate host. Methods: Two cynomolgous macaques (Macaca fascicularis) were infected with virulent feline immunodeficiency virus (FIV, Petaluma strain). This was achieved by first infecting recovered peripheral blood mononuclear cells (PMBC) with the FIV, allowing three days for viral replication in vitro and returning 4 x 10^6 PMBC to the same donor by intravenous transfusion. Animals were monitored for 12 weeks. One animal received tetanus toxoid at 9 and 10 weeks postchallenge. The course of infection was monitored by recording body weight changes, blood cell enumeration and typing. Infection was also monitored by serology for anti-FIV antibodies and FIV-specific sequences by nested PCR amplification and Southern blot detection of the FIV pol gene. At 12 weeks postinfection animals were euthanized and tissues collected to demonstrate the presence of virus. Results: FIV infected cynomolgous macaques exhibited weight loss and a 76.5% decrease in CD4+ lymphocytes (P < 0.05). The development of an antibody response to FIV proteins and the detection of virus specific sequences in sera and blood-derived cells accompanied these changes. FIV specific sequences were detected in the spleen and lymph nodes at the time of necropsy, 12 weeks postinfection. Conclusion: This study suggests that the use of lentiviruses in human gene therapy of nonhuman cells generates by mice in the standard unenriched environment, and those bred and weaned in an unenriched environment, and then switched to an enriched one.

We measured the following parameters of immunity: thymus size and distribution of lymphocyte subsets, cytokine responses to immunization with ovalbumin, antibody isotype responses to ovalbumin, and the response to malaria infection. We found that the group of mice that were switched to an enriched environment had dramatically smaller thymuses than those that were not switched, and that the depleted thymocyte population was primarily immature (CD4+CD8+) cells. This result is consistent with the effects of endogenous glucocorticoid release.

None of the other parameters examined were significantly influenced by environmental enrichment. The elaboration of IL-2, IL-4, IL-10 and IFNg in response to ovalbumin was similar in the two groups, and production of immunoglobulin isotypes (IgG1, 2a, 2b and 3) was not affected. Finally, there was no difference between the groups in their ability to control malaria infection. Our data suggest that while the immune response to one commonly used model antigen, ovalbumin, is not affected by environmental enrichment, enrichment does have a profound effect on the thymus; therefore, placing mice intended for the study of T-cell development in an enriched environment should be carefully considered.

**PS10 The Effects of Environmental Enrichment on Murine Immune Responses**

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Much of our understanding of the mammalian immune system comes from studies using laboratory mice. All aspects of immunity, including the development of the immune system, responses to simple antigens, complex infectious diseases and autoimmunity, have been extensively modeled in this species.

In an effort to provide a higher quality of life for our experimental mice, many of which are used to study immunity to parasitic infectious diseases, our facility decided to offer various forms of environmental enrichment, including nestlets and jars. Although the effects of environmental enrichment on parameters of immunity have not been extensively assessed, some studies have shown that enrichment can be a stressor, and stress has profound effects on immune responses. We tested the hypothesis that immune responses generated by the mice in the enriched environment would be qualitatively different than those generated by mice in the standard unenriched environment, and that those responses would reflect the effects of stress on the immune system.

Our original study design used four groups of BALB/c mice—two bred and raised to weaning in an unenriched environment and two in an enriched environment. One group from each of these treatments was then to be switched to the alternative environment. We found, however, that the breeding pairs in cages that included enrichment (nestlets, jars, a ladder) had significantly fewer litters than the pairs in the cages that did not (P < .01). Because of the small numbers of mice raised in an enriched environment, the immunologic studies were carried out with two groups of mice; those bred and weaned in an unenriched environment, and those bred and weaned in an unenriched environment and then switched to an enriched one.

We measured the following parameters of immunity: thymus...
PS12 Comparison of Sentinels and Reverse Transcriptase Polymerase Chain Reaction to Assess Shedding of Mouse Hepatitis Virus Y from Immunocompetent Mice

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Duration of mouse hepatitis virus (MHV) shedding and transmission from immunocompetent mice has been reported anecdotally to vary greatly. To assess sensitivity of contact sentinels and reverse-transcriptase polymerase chain reaction (RT-PCR) for detecting MHV shedding, four cages of four 3- to 4-week-old Helicobacter-free female BALB/c mice were orally inoculated with enterotropic MHV-Y. One day postinoculation (dpi), index mice were transferred to clean cages containing an adult female Swiss Webster mouse (contact sentinel). Three dpi, feces were collected from index mice prior to transfer to a clean cage containing a new contact sentinel. Contact sentinels were held in soiled cages for a week, then tested for MHV seroconversion by immunofluorescence assay at 2 weeks postexposure. Mouse transfers and feces collections were performed at 2- to 5-day intervals through 77 dpi. Feces were tested by RT-PCR using MHV N gene primers. While duration of MHV shedding varied from 27 to 62 days, seroconversion of contact sentinels correlated with MHV RT-PCR results confirming that infectious viral RNA was detected. All animals were Helicobacter negative by PCR. A second experiment investigated the effect of index mouse genotype on MHV transmission to sentinels exposed to soiled bedding for 7 days or in contact with index animals for 1 day plus soiled bedding for 6 days over a 49-day period. Infected BALB/c mice (n = 12) transmitted MHV to contact sentinels (n = 21) for 28 or 35 days. Soiled bedding from infected BALB/c mice (n = 12) transmitted MHV to sentinels (n = 21) for up to 27 days, whereas soiled bedding from infected C57BL/6 mice (n = 12) transmitted MHV to sentinels (n = 21) for 15 days. These results indicate that MHV shedding is influenced by mouse genotype, and contact and bedding sentinels consistently detect MHV shedding. One possible explanation for the extended shedding by index mice in the first experiment could be the continuous presence of naive contact sentinels in the first but not the second experiment.

PS13 Pathogenesis of Enteric Mouse Hepatitis Virus Strain Y in Helicobacter-free Immunocompetent and Immunodeficient Mice

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Previous studies described the pathogenesis of enterotropic MHV during a time when mice were endemically infected with Helicobacter. To confirm and further characterize MHV-Y pathogenesis, 40 adult female Helicobacter-free BALB/c mice were inoculated orally with MHV-Y and assessed through 29 days postinoculation (dpi). Feces were positive and blood was negative for MHV RNA by reverse-transcriptase polymerase chain reaction (RT-PCR) through 29 dpi. Seroconversion occurred by 8 dpi and no gross or microscopic lesions were detected through 29 dpi. In situ hybridization (ISH) detected MHV RNA in the small intestines, mesenteric lymph nodes and Peycer’s patches on 2, 3 and 5 dpi and in ceca and colons through 15 dpi. To examine the role of host immunity in MHV-Y pathogenesis, 22 adult fe-

PS14 Characterization of a Novel Parainfluenza Virus, Caviid Parainfluenza Virus 3, from Laboratory Guinea Pigs (Cavia porcellus)

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A novel Respirovirus was isolated from throat swabs of clinically normal guinea pigs, characterized, and named caviid parainfluenza virus 3 (CavPIV-3). CavPIV-3 is enveloped, 100-300 nm in diameter, and has a characteristic 15 nm diameter chevron-shaped virus ribonucleocapsid protein. Sequence analysis of the fusion glycoprotein of CavPIV-3 revealed that it is 94% identical to both human and guinea pig parainfluenza 3 (PI-3) viruses, and that it is 80% identical to bovine PI-3 virus. To determine whether CavPIV-3 causes clinical disease in laboratory guinea pigs and to compare the serologic response of guinea pigs to CavPIV-3 and to other paramyxoviruses, an infection study was performed. In the infection study, groups of guinea pigs were inoculated with CavPIV-3, Sendai virus, simian virus 5 (SV-5), murine pneumonia virus, and bovine PI-3 virus. During the course of the study, guinea pigs were maintained in an infectious disease suite, housed in microisolator cages, and were only manipulated in a laminar flow hood. No clinical signs of disease were noted in any of the guinea pigs during the 8-week course of the study, and no histologic signs of disease were noted at necropsy 8 weeks postinoculation. Guinea pigs inoculated with CavPIV-3, Sendai virus, murine pneumonia virus, and bovine PI-3 virus developed robust homologous or heterologous serologic responses. In contrast, guinea pigs inoculated with SV-5 only developed modest or equivocal serologic responses as assessed by enzyme linked immunosorbent assay. Further, the SV-5 enzyme linked immunosorbent assay resulted in the highest degree of non-specific reactivity among all of the paramyxovirus assays. In summary, CavPIV-3 is a novel guinea pig Respirovirus that subclinically infects laboratory guinea pigs, resulting in a robust serologic response, but no observed histologic disease. The CavPIV-3 fusion glycoprotein gene sequence is available from GenBank as accession AF394241.
ELISAs are the most popular test format to monitor pathogen exposure in mouse facilities. Panels of up to 20 different ELISAs are used. We have developed an immunoarray that can perform simultaneous detection of >20 pathogens on a 20 µl serum sample. Using a new, flexible, microarray system, we have developed and are validating an assay to simultaneously detect murine antibodies directed against mouse hepatitis virus (MHV), epidemic diarrhea of infant mice (EDIM), minute virus of mice (MMV), and mouse parvovirus (MPV). The microarrays were prepared using viral proteins from cellular extracts (MHV, EDIM) or recombinant protein antigens (MPV, MVM) purchased from the University of Missouri Research Animal Diagnostic Laboratory. Controls included cell culture controls, negative controls (bovine serum albumin) and a positive mouse immunoglobulin control. Protein is adsorbed in bulk to a colloidal porous three-dimensional structure of the spots, which results in a very high signal-to-noise ratio due to the porous threedimensional structure of the spots, which results in an extremely high surface area.

**PS16 Development of a Highly Sensitive and Specific Fluorogenic Probe PCR Assay for the Detection of Mycoplasma pulmonis**

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*Mycoplasma pulmonis* is the causative agent of murine respiratory mycoplasmosis, a disease of considerable importance in laboratory rats and mice. In order to provide a rapid method to monitor for its presence, we have developed a fluorogenic probe (Taqman) PCR assay for the detection of *M. pulmonis*-specific nucleic acids. Based on an alignment of 16S ribosomal RNA gene sequences from numerous mycoplasmas and other bacteria, PCR primers were designed that would permit specific amplification of an 81 base-pair product from *M. pulmonis* DNA. An oligonucleotide probe (labeled with a fluorophore and a quenching dye) that anneals to this product was also designed. As the PCR reaction proceeds, the fluorophore is separated from the quencher, permitting the detection of amplification directly in the reaction tube by fluorimetry without the need for gel electrophoresis. This primer/probe combination was capable of amplifying DNA isolated from a culture of *M. pulmonis*, while DNA from mammalian cell lines and other mycoplasma species was not detected by the assay. A positive control template consisting of the amplified DNA fragment cloned into a plasmid vector was constructed.

In analytical sensitivity experiments, the 50% endpoint for detection by the assay (as determined by the Reed-Muench method) was calculated as 2.2 copies of the control template—equivalent to 8.4 attograms (10⁻¹⁸ g) of DNA.

**PS17 Recombinant MPV VP2 Truncated Proteins as Antigen for Parvovirus ELISA**

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MPV VP2 and MVM VP2 truncated and full-length proteins from Charles River Labs (CRL), Yale University (Yale) and University of Missouri (UMO) were compared for their efficacy to detect the MPV VP2 and MVM VP2 antibodies in rodent sera.

Recombinant MPV VP2 truncated proteins for detection of parvovirus were cloned using the Gateway cloning and expression system. N- and C-terminal proteins with His tag were produced in BL21-SI bacterial cells. The expressed truncated proteins were purified individually from cell lysates using nickel-chelating chromatography procedure. Purified antigens MPV VP2 (N) and MPV VP2 (C) were qualified and pooled together. This mixture of proteins was used as antigen for coating the 96-well plates for MPV VP2 (CRL) ELISA. Field trials were run using 440 mouse sera samples from serology. Results from MPV VP2 antigen from CRL and Yale were comparable with no false positives. In addition, mouse sera samples positive for MVM VP2 and MPV VP2 antibodies were tested by MPV VP2 antigens from CRL, Yale, UMO and MVM VP2 (UMO) antigen. Out of 29 positive tested samples, only 21 were confirmed positive by CRL and Yale MPV VP2 ELISA. MPV VP2 (UMO) ELISA showed 15/29 positives and MVM VP2 (UMO) antigen showed 29/29 positives.

Results from the field trials suggested that UMO antigens were more specific in detecting MVM or MPV antibodies than CRL and Yale antigens.

**PS18 Serodiagnosis of Mice Minute Virus and Mouse Parvovirus Infections in Mice by ELISA using Baculovirus-Expressed Recombinant VP2 Proteins**

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Mice minute virus (MMV) and mouse parvovirus-1 (MPV) are the two parvoviruses known to naturally infect laboratory mice and are among the most prevalent infectious agents found in contemporary laboratory mouse colonies. Serologic assays are commonly used to diagnose MMV and MPV infections in laboratory mice; however, highly accurate, high-throughput serologic assays to detect MMV- and MPV-infected mice are needed. To this end, the major capsid viral protein (VP2) genes of MMV and MPV were cloned and MMV recombinant VP2 (rVP2) and MPV rVP2 proteins were expressed using a baculovirus system. MMV rVP2 and MPV rVP2 spontaneously formed virus-like particles that were morphologically identical to empty parvovirus capsids. These proteins were used as antigens in ELISAs to detect anti-MMV or anti-MPV antibodies in the sera of infected mice. Sera from mice experimentally infected with MMV (n = 43), MPV (n = 35) and sera from uninfected mice (n = 30) were used to evaluate the ELISAs. The MMV ELISA was 100% sensitive and 100% specific in detecting MMV-infected mice and the MPV ELISA was 100% sensitive and 98.6% specific in detecting MPV-infected mice.
Helicobacter bilis infection is widespread among research mouse colonies. Infections are usually subclinical, but hepatic and enteric disease has been reported in certain strains of mice and mice with immunodeficiencies. The diagnosis of H. bilis infection can be made by culture, PCR and serology. Serodiagnosis of Helicobacter infections uses bacterial lysates or membrane antigen preparations that lack specificity, necessitating the need to identify a specific and sensitive antigen for the serodiagnosis of H. bilis. We have reported on novel recombinant H. bilis gene products (P167C and P167D) that were identified by serologic screening of an H. bilis genomic expression library. These proteins were determined to be immunodominant and specific for H. bilis. The P167C and P167D recombinant proteins were evaluated for the use as an H. bilis-specific antigen in the serodiagnosis of mice naturally infected with H. bilis. Eighty-one mice naturally infected with Helicobacter were identified from commercially bred or sentinel mice. Infection was confirmed and speciated by cecal culture and/or PCR followed by restriction enzyme digest of the amplicon. Forty-six mice were determined to be monoinfected with H. bilis and 35 mice were monoinfected with H. hepaticus. Serum was diluted 1:100 to evaluate the immunoreactivity to dot blot preparations of H. bilis membrane extract and recombinant P167C and P167D proteins. The sensitivity was greatest for the membrane extract preparation (94%); however, the specificity was low (31%). The sensitivity of the recombinant P167C and P167D protein was less than the membrane extract (70% and 74%, respectively), and the specificity of the P167C fragment was higher than the P167D fragment (94% and 66%, respectively). Using a combination of the two recombinant proteins the sensitivity was less than the membrane extract (67%), and the specificity was similar to that when using p167C alone (94%). Cross-reactivity occurred with sera from mice naturally infected with H. hepaticus to the H. bilis membrane extract, and to the P167C and P167D proteins with a false positive incidence of 69%, 6% and 29%, respectively. Similar findings were seen in ELISA-based preparations. These findings suggest that the recombinant P167C and P167D proteins from H. bilis can be used as a specific test in the serodiagnosis of H. bilis infection in mice.

PS19 Specificity of a Recombinant Antigen in the Serodiagnosis of Helicobacter bilis

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Prevalence of these parasites may be as high as 100% in some animal species. Recognition of zoonotic genotypes is necessary to determine human health risks when handling animals that may be potentially infected. Methods: Cryptosporidium spp. were isolated from the feces of naturally infected dogs, cats, pigs, and calves and adult cattle from either animals within the animal resource centre or local suppliers. These were genotyped by sequence analysis of the HSP-70 loci. Cryptosporidium isolated from over 150 infected humans in the local health region were also genotyped. Cross-species transmission to decamethone immunosuppressed mice (C57BL) and rats (Sprague Dawley) was attempted by orogastric inoculation of approximately 10^7 oocysts. Animals were monitored for cyst shedding and intestinal colonization for up to 3 weeks postchallenge. Results: Cryptosporidium spp. isolated from dogs, cats and pigs was unique to their respective species. Young calves were infected with the zoonotic genotype (Type 2) while older calves were infected with Cryptosporidium andersoni. The human population was predominantly infected with human genotype (Type 1) but approximately 20% were infected with zoonotic genotype (Type 2). C. felis was detected in two immunocompetent children and C. meleagrisidis was found in the feces of two travelers. Cross-species transmission was successful between Type 2 genotype and rodents (mice and rats). Conclusion: Cryptosporidium is a common protozoan parasite in certain laboratory animal species. The Cryptosporidium spp. carried by most laboratory animals pose a minimal zoonotic risk, while others (e.g., calf Cryptosporidium) poses a serious zoonotic risk. Type 1 Cryptosporidium is not infectious to immunocompetent laboratory rodents but Type 2 Cryptosporidium may infect most laboratory rodents.

PS21 Effect of Bedding Sterilization and Type on the Incidence of Urogenital Disease in the Estrogenized Nude Mouse

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The estrogenized nude mouse serves as a popular model for breast cancer studies. Mice are implanted with continuous-release estrogen pellets, to support the growth of several estrogen-dependent breast cancer cell lines. In this study, estrogenized, tumor-implemented mice developed severe urogenital disease that resulted in premature death before the tumors were ready for harvest. Disease manifestation ranged from perineal scalding to pyelitis to cystitis. Because chronic estrogen exposure has been associated with decreased muscular contraction of the urinary bladder and subsequent urinary retention, we hypothesized that mice undergoing chronic estrogen exposure are more susceptible to ascending infections of the urinary tract and examined whether changes in bedding that might decrease perineal bacterial colonization would influence the incidence of severe urogenital disease in this model. Groups of NCR nu/nu mice were implanted with 0.36 mg estrogen pellets and maintained on sterilized paper bedding. These animals were necropsied at 9 weeks postestrogen pellet implantation or at onset of clinical disease. The prevalence of urogenital disease in mice originally maintained on non-sterilized corncob bedding was approximately 80% with symptoms beginning around 5-6 weeks postimplantation. In contrast, in mice maintained on paper bedding, the prevalence of disease was less than 20%. Changing the bedding to sterilized paper bedding has allowed for the maintenance of animals until tumor harvest can be performed. In conclusion, estrogenized mice present a challenge to maintain as a result of increased incidence of urogenital disease. However, our results suggest that a change in bedding effectively

PS20 Cryptosporidium spp. in Laboratory Animals and Their Potential Zoonotic Risk

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Parasites of the genus Cryptosporidium are a significant and widespread cause of enteric disease in humans and animals.
decreases the incidence of clinical disease in these mice and allows maintenance of mice for the extended periods of time necessary for completion of certain studies.

**PS22 A Report of *Burkholderia gladioli*, a Vancomycin-Resistant Opportunistic Pathogen, in Immunocompromised Mice**

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In May 2001, an athymic nude mouse presented with a severe head tilt due to otitis interna. Culture of the exudate was initially identified as *Burkholderia cepacia*, a plant pathogen considered an important opportunistic pathogen in persons with cystic fibrosis or chronic granulomatous disease. Within 1 week, more immune-deficient mice with severe (90°) head tilts were identified from the same suite of mouse barrier rooms, and within 3 weeks a variety of immune-deficient and mice from other facilities were presented for necropsy with head tilts. In all cases, otitis interna was the only grossly observed lesion. Clinically, mice would spin intensely when elevated by the tail, and begin rolling to the side of the lesion when placed back in their cage. Several bacterial isolates sent to the University of Michigan *B. cepacia* Research Laboratory & Repository were identified as *Burkholderia gladioli* based on biochemical analysis and species-specific PCR assay. A 16S rDNA-based PCR assay specific for the genera *Burkholderia* and *Ralstonia* was used in efforts to characterize the epidemiology of this organism. Samples analyzed from affected mice included the environment, oropharyngeal cavity, feces, sipper tubes, drinking water, and soiled bedding from cages of affected individuals. In addition, a selective media containing vancomycin, polymyxin B and gentamicin was used to isolate colonies of immune deficient mice both by PCR and culture on selective medium. PCR was equivalent to selective culture in identifying asymptomatic mice in the carrier state. Mice that were affected by the organism included SCID.Bg, SCID.NOD, C3H.SCID, Rag-1/-, Balb/c nu/nu, C57Bl/6 nu/nu, COX-1/-, TCRα/-, and Tg[TcReta]. Identification of carrier mice was attempted; however, sensitivity of culture and PCR were not 100%, causing the organism to persist at low levels unless entire colonies of immune deficient mice were removed. The organism was highly resistant to antibiotic therapy. The source and epidemiology of this organism remain unknown. This epizootic serves as an important reminder that immunocompromised rodents may harbor significant human opportunistic pathogens.

**P23 Metal Ear Tag-Induced Foreign Body Tumorigenesis in p53-/- Mice**

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During the course of a chronic inhalation study with the water disinfection by-product bromochloromethane (BDCM), a high incidence of soft-tissue sarcomas arose on the pinnae of the ears of mice heterozygous for the p53 tumor suppressor gene (p53+/−). Mesenchymal neoplasms (fibrosarcomas) in this unusual location were closely associated with the presence of a metal ear tag and were not related to the chemical exposure. This chronic toxicity/oncogenicity study employed 150 p53+/- mice on the C57BL/6 genetic background and 150 mice on the FVB/N background. A substantially higher sarcoma incidence, shorter latency to onset, and more rapid tumor growth was noted in mice on the FVB/N genetic background. Animals were ear-tagged with metal implants between 8 and 10 weeks of age and then maintained on study for an additional 12 months. About 40% of the p53-/- FVB/N mice developed ear sarcomas during the course of the study. The first tumor was noted at about 6 months following ear-tag implantation. Many of the sarcomas in the p53-/- FVB/N mice were locally aggressive neoplasms that rapidly invaded local structures and developed ulcerated surfaces, necessitating unscheduled early removal of tumor-bearing animals from the study. In contrast, the p53-/- C57BL/6 mice developed fewer than a 5% incidence of slow growing sarcomas associated with the metal ear tags. These observations emphasize the need to carefully consider routine husbandry procedures when conducting chronic studies with genetically modified mouse models. The p53-/- mouse on an FVB/N genetic background may represent a new and useful animal model for studies of implant-associated sarcomagenesis.

**PS24 Management of Intraoperative and Postoperative Complications in a Coronary Artery Surgery Model in Sheep**

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Background: The sheep is considered a suitable model for cardiovascular surgery because of its ease of handling, size and vascular anatomy, which bears close resemblance to humans. Several difficulties, however, have limited the use of the sheep for such a purpose, mainly high infection rate of a median sternotomy incision and a susceptibility to intractable ventricular fibrillation with the slightest manipulation of the heart and/or short periods of myocardial ischemia. We have used the sheep model extensively to perform coronary artery bypass surgery and were successful in overcoming these difficulties.

Methods and results: Fifty-seven adult female sheep were used to test a new anastomotic device for the creation of a sutureless connection between venous and arterial grafts and the coronary arteries. The study required full access to the heart and great vessels and mobilization of one of the internal mammary arteries. Changing to the left lateral thoracotomy approach solved initial fatal problems of postoperative infected median sternotomy incisions. Aggressive prophylactic treatment with anti-arrhythmic drugs, maintenance of normothermia and myocardial preconditioning rendered the heart much less vulnerable to manipulations and ischemia. These measures have reduced the mortality rate from 45% to 0% (P < 0.0001). Conclusion: With specific operative techniques and pharmaceutical interventions, the sheep can effectively and safely be used as a model for coronary artery surgery.

**PS25 Cyclic Hematuria and Anemia in a Sheep**

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A 10-month-old male castrated domestic sheep (*Ovis aries*) presented with hematuria. The animal had arrived 1 month...
previously from a Class A vendor, and physical exam, CBC and chemistry performed on arrival were normal. It was group housed in an enclosed paddock and was fed pelleted ruminant diet and oat hay cubes with water ad libitum. The animal was treated pre- sumpitively with penicillin for a urinary tract infection. Blood and urine exam results showed anemia (HCT 15%), hemolysis and hematuria (4+ RBCs). The hematuria resolved within 7 days. Potential diagnoses included copper toxicity, urinary tract disorders, immune-mediated hemolysis and blood parasites. Copper and lead levels were normal. No parasites were noted on blood smears. Three weeks later, the animal again presented with hematuria and anemia and was treated with PenG, iron and B vitamins. The hematuria resolved within 6 days. Serial blood samples and smears were collected to monitor HCT and examine red cell morphology. Again 3 weeks later the animal presented with hematuria and anemia. The serial blood smears were sent in for analysis; many *Eperythrozoon ovis* ring forms were identified on multiple smears collected between anemic episodes. The animal was treated with a single dose of oxytetracycline intramuscularly plus iron complex. Initially, the animal’s HCT recovered but there were additional episodes of anemia at 4 and 8 weeks following treatment. At the 8 week reocurrence the animal was re-treated with oxytetracycline (20 mg/kg) daily for 10 days. The animal’s HCT returned to normal and it gained approximately 10 kg over 2 months. However, at 10 weeks post-treatment the animal was again anemic and was re-treated with high dose oxytetracycline for 10 days. Four weeks later, the animal’s HCT was 35% and it appeared in good health. It was assigned to an acute study and was euthanized. Gross necropy showed no significant findings. Samples were submitted for his- topathology and further confirmatory diagnostic tests. Few references describe *E. ovis* infection in the United States and there is disagreement over its clinical significance and its taxo- nomic designation as Rickettsia versus Mycoplasma. *Eperythrozoon ovis* should be considered as a rule-out for anemia in sheep; it is important to examine blood smears taken prior to a clinical epi- demic of anemia or in the recovery period as parasites may be impossible to find in the severely anemic samples.

**PS26 Hemorrhagic Pneumonia Due to Necrotoxigenic *E. coli* in Three Beagle Dogs**

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*Escherichia coli* has been documented as a frequent pathogen in animals and humans, both as a cause of enteric disease and extraintestinal infections. Over a 9-month period, three Beagle dogs at our facility suffered fatal, peracute pneumonia. In each case, *E. coli* was isolated from the lungs. *Streptococcus* group G was also isolated from the lung of one dog. Tests for canine parain- fluenza virus and canine adenovirus on lung tissue were negative. The dogs came from two different vendors, and in each case the affected dog had recently been shipped to our facility. The dogs had been administered the standard canine vaccines at the vendor prior to shipment, including vaccination against *Bordetella bronchiseptica*. The cases presented as a peracute clinical syndrome and appeared histologically as a hemorrhagic pneumonia. In cases of pneumonia, *E. coli* organisms are felt to reach the lung tissue either by aspiration of oropharyngeal secretions or through hematogenous dissemination from a primary source, most likely the gastrointestinal or genitourinary tract. *E. coli* was also isolated from blood in all three of these cases. Serotyping of the *E. coli* isolates indicated that two were O6 and one was O4. The O4 isolate had the same ribotype pattern as one of the O6 isolates, indicating genetic similarity. Isolates from all three dogs were positive for the virulence factor cytotoxic necrotizing factor 1, placing them in the class of necrotoxigenic *E. coli* (NTEC). A 1987 study in people found 40% of *E. coli*-associated extraintestinal infections to be due to necrotoxigenic *E. coli*, whereas less than 1% of the *E. coli* strains isolated from the stool of normal people were found to be necrotoxigenic. A 1997 study in dogs found 52% of *E. coli*-associated urinary tract infections to be due to necrotoxigenic strains. Thus, NTEC are being in- creasingly implicated as a cause of extraintestinal infections in animals and humans.

**PS27 Removal of Feral Swine from Ossabaw Island and the Chal- lenges in Establishing a Herd Health Program**

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Feral swine from Ossabaw Island, Georgia, were captured and removed to establish a breeding colony at the University of Miss- ouri—Columbia. Ossabaw swine are genetically predisposed to develop obesity and Type II diabetes, and have the potential to be an important model to study this disease. Several challenges were encountered in the removal and establishment of a healthy colony of feral swine. Animals on the island were known to be endemically infected with Brucellosis (BR), pseudorabies (PRV) and vesicular stomatitis (VS-IN and NJ serotypes). Federal and state (Missouri and Georgia) regulatory agencies agreed upon a plan for removal of animals from the island. A total of 104 pigs were captured and tested for BR, PRV, and VS. Only animals serologically negative for all three agents were allowed off the island. Swine negative for these diseases were immediately transported to Missouri for a quarantine period and are awaiting further testing. Results of serologic testing revealed an incidence of infection of 39.4% for PRV, 6.7% for BR and 71.2% for VS. Twenty adult males were tested and 100% of these were infected with at least one of the agents. Twenty-six seropositive pigs of various ages and both sexes were transported to Missouri. Ex- amination of multiple fecal samples revealed heavy loads of *Acaris suum*, *Strongyloides ransomi*, *Hystrostrongylus rubidus*, *Isospora suis*, *Eimeria spp.*, *Trichuris suis*, *Metastrongylus apri*, and *Oesophago- gestumum dentatum*. Ectoparasites also were evaluated and revealed numerous ticks (*Amblyomma* spp) and lice (*Haematopinus* spp.). Feral swine required a unique herd health program, due to their previous environmental isolation, diet and their relatively un- known health status. The establishment of a breeding herd of Ossabaw feral swine for use in biomedical research brought about new challenges. Through proper quarantine and herd health programs, research institutions can overcome the challenges associated with the importation of feral animals.

**PS28 Typhlocolitis and Uremic Syndrome in Rabbits Coinfected with *Clostridium difficile* and Enteropathogenic *Escherichia coli***

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Three out of six 10-week-old Dutch belted rabbits recently received from a commercial vendor developed severe watery diar- rehea and lethargy. One of the animals died acutely. A complete
blood count performed on one rabbit with hematochezia revealed mild anisocytosis, poikilocytosis, and thrombocytopenia. Serum chemistries revealed prominent elevations in BUN, creatinine, and liver enzymes in both diarrheic rabbits. Numerous epithelial cells, hyaline and granular casts, protein, and red blood cells were detected by urinalysis.

*Escherichia coli* of the same biotype was cultured from the cecal contents of two of the affected rabbits. This *E. coli* isolate was serotyped as O145:H1- and PCR analyses amplified intimin (eae) and lymphostatin (lif/A), but not shiga toxin sequences. Lymphostatin, a recently described virulence factor that bears significant homology to the *C. difficile* toxins, was confirmed by sequence analysis. Interestingly, enterohemorrhagic *E. coli* (EHEC) strains such as O157:H7 carry a similar gene in the large plasmid (pO157). The *C. difficile* Tox A/B ELISA was also positive in these two affected rabbits and cell culture assay confirmed toxin B activity in the cecal contents of one of them. PCR analyses amplified *C. difficile* in the cecal contents of one affected rabbit and in three out of four clinically unaffected Dutch belt rabbits from the same colony; however, the *C. difficile* Tox A/B ELISA and cell culture assay were negative in all four controls. In addition, fecal cultures revealed that all the controls harbored a nonpathogenic O7:H- *E. coli*. Histological lesions in all affected rabbits included typhlolocitis with adherent bacterial rods, nephrosis, and fibrin deposition in the glomerular capillaries. These findings suggest that *C. difficile* and enteropathogenic *E. coli* (EPEC) may act synergistically, inducing a disease phenotype akin to hemolytic uremic syndrome (HUS).

**PS29 Effect of Pair Housing on Operant Behavior Task Performance by Rhesus Monkeys**

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Social housing is the method of choice for nonhuman primates who live in social groups in the wild. However, social housing can affect physiological and behavioral parameters. This study evaluated the effects of pair housing on several operant (trained) behaviors in rhesus macaques (*Macaca mulatta*). Sixteen young, male, individually housed, trained rhesus monkeys (2.5-5.5 years of age) performed a battery of behaviors consisting of motivation (MOT), short-term memory and attention (STM), color and position discrimination (CPD), and learning (LRN) tasks. Behavioral assessments occurred five days/week with the LRN, MOT, and CPD tasks presented on one test day and the STM task presented on the alternate test day. Thus, each task was performed two or three days/week. The subjects were divided into four age cohorts, and within each cohort two randomly selected subjects were pair housed, while eight age-matched controls remained individually housed. Pair-housed monkeys were separated for behavior testing and feeding, but allowed access to each other approximately 20 h/day. The performance of the two groups of monkeys were compared for the 2 months prior to pairing, for a 2-month transition period as the pairs adjusted to the new housing situation, and for a 2-month period after the pairs had been established. Performance in the CPD and LRN tasks did not change over time in either group. For the MOT and STM tasks, the number of trials completed increased over the course of the study in the controls, but not in the pair-housed monkeys. Thus, pair housing may have no affect on some operant behaviors while affecting others in the same subjects. This possibility should be taken into consideration during determination of housing options.

**PS30 Paint Roller and Grooming Boards as Treatment for Over-grooming Rhesus Macaques**

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Laboratory monkeys living in cages can develop atypical behaviors believed to indicate diminished psychological well-being. One of the most pervasive is self-directed over-grooming. Some monkeys groom their own hair to the point that they are almost bald. Typically, monkeys who exhibit this behavior are given grooming boards (a metal frame with fleece covering hung outside of the cage), with the hope that the behavior will be re-directed onto them. However, these devices are time-consuming to assemble, and alternatives are desirable. One such device is a paint roller placed over a PVC pipe hung horizontally outside of the cage. In this study, we compared the efficacy of grooming boards and paint rollers in the treatment of over-grooming. The subjects were 18 rhesus macaques with behavioral diagnoses of over-grooming. The monkeys were randomly placed into one of three groups, and received either grooming boards covered in a mixture of crackers and honey (n = 6), paint rollers rolled in the same mixture (n = 6), or no device (controls, n = 6). Once a week, two behavior technicians assessed the percent hair loss for each monkey, and replaced the devices. All animals, including controls, received new toys each week, along with daily produce provided for increased foraging opportunities. The monkeys have been followed for 6 weeks. The assessments were highly concordant between the technicians (r = 0.89, P < 0.001); therefore we used the average score for analyses. We calculated an ‘improvement score’ as score for week 6 minus the score for week 1. We found no differences among treatments with respect to this improvement score (Kruskal-Wallis = 1.29, P > 0.5). In each group, most animals showed little, if any, improvement. Thus, our preliminary data suggest that neither grooming boards nor paint rollers affect over-grooming behavior after 6 weeks. More work is needed to determine whether there will be differences in the long term.
ages to anti-DNA antibody-free IFN-γ−/− mice did not induce autoantibody production, but provoked upregulation of adhesion molecules as well as a notable infiltration of macrophages to the kidney interstitium, indicating that the source of IFN-γ contributing to local inflammation is not kidney-resident cells, but infiltrating macrophages. Equivalent transfer of T cells to IFN-γ−/− mice did not result in kidney inflammation, further confirming that macrophage-produced IFN-γ is essential for this process. Our findings demonstrate that, independently of autoantibody deposition, autocrine IFN-γ production by infiltrating macrophages is responsible for adhesion molecule upregulation, macrophage accumulation and propagation of inflammation in the kidney. These results outline the pathway of the inflammatory process in lupus and may be useful in designing treatment for disease, even after autoantibody development.

**PS33 Determination of Maximum Tolerated Dose Levels of Carboplatin, Fluorouracil, Irinotecan HCl, Paclitaxel, and Doxorubicin HCl in SCID Mice**

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Carboplatin, fluorouracil, irinotecan HCl, paclitaxel, and both doxorubicin HCl and doxorubicin HCl liposome injections are potentially useful controls for human xenograft studies in severely immunodeficient mice. Since tolerability information in C.B-17-SCID mice is currently unpublished, a dose-range finding tolerability study was conducted. Female C.B-17-SCID mice were 4-6 weeks old and 18-22 g at study initiation. Three to five mice per dose level were assigned arbitrarily to groups. Clinical observations were performed daily. Body weights were measured twice weekly. Tolerability was defined by a less than 10% body weight loss from the time of dosing initiation and the absence of adverse clinical observations, such as labored respiration, prostration, cessation of eye, facial edema, lethargy, inactivity. Initial doses and regimens were based on previously published information with nude mice. Subsequent doses were administered consecutively to naïve mice and increased or decreased based upon the tolerability of previous doses. Vehicle controls received 5% dextrose for injection. Adverse clinical signs and/or body weight loss in excess of 10% were observed at the following dose levels: Carboplatin at 100 mg/kg after a single dose, fluorouracil at 100 mg/kg and above after 2 twice-weekly doses, paclitaxel at 30 mg/kg and above after a single dose, doxorubicin HCl at 5 mg/kg and above after a single dose, and doxorubicin HCl liposome injection at 4 mg/kg after 4 weekly doses. Based on the results of this study, the following maximum tolerated dose levels were determined: Carboplatin 75 mg/kg once weekly for 4 weeks, fluorouracil 50 mg/kg twice weekly for 4 weeks, irinotecan HCl 30 mg/kg daily for 5 days, paclitaxel 20 mg/kg once weekly for 4 weeks, doxorubicin HCl 1 mg/kg single dose and doxorubicin HCl liposome injection 2 mg/kg once weekly for 4 weeks.

**PS34 Efficacy of Carboplatin, Fluorouracil, Irinotecan HCl, Paclitaxel, and Doxorubicin HCl Liposome Injection against Human Tumor Xenografts in SCID Mice**

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Carboplatin, fluorouracil, irinotecan HCl, paclitaxel, and doxorubicin HCl liposome injections are potentially useful controls for human xenograft studies in immunodeficient mice. Since therapeutic dose information in C.B-17-SCID mice is currently unpublished, efficacy studies were conducted with the following cell lines: MDA-MB231 (breast), MCF7 (breast), C8161 (melanoma), NCH-H460 (colon), HCT116 (colon), PC3 (prostate) and ES-2 (ovarian). C8161, HCT116, PC3, NCH-H460 and ES-2 cells were implanted subcutaneously in the flank at 1×107 cells/mouse. MCF7 and MDA-MB231 cells were implanted in the mammary fat pad at the same cell concentration. MCF7 cell growth was supplemented with 17β-estradiol. Female C.B-17-SCID mice were 46-weeks old and 18-22 g at study initiation. Treatment was initiated in mice with established tumors (50-100 mm3). Tumor volumes were measured twice weekly for 5 weeks and group means compared with untreated control groups. Treatment doses were administered intraperitoneally at 10 mg/kg. Compared to controls, carboplatin, administered at 75 mg/kg on Day 3 after tumor implant followed by 20 mg/kg weekly thereafter, was ineffective against C8161 tumors. Weekly 20 mg/kg-doses were likewise ineffective against H460 and ES-2 tumors. Fluorouracil was shown to have a significant growth-inhibitory effect on HCT116 tumors at twice-weekly doses of 30 mg/kg as compared to controls (P < 0.0005). Irinotecan was significantly efficacious against MCF7 (P < 0.05), HCT116 and H460 tumors after five consecutive, daily doses of 20 mg/kg (P < 0.000002). Paclitaxel was also significantly efficacious against HCT116 and PC3 tumors at weekly 20 mg/kg doses, yet was ineffective against MCF7 and H460 tumors (P < 0.0002). As compared to controls, doxorubicin HCl liposome injection demonstrated a significant growth-inhibiting effect against MDA231, MCF7 and H460 tumors at weekly doses of 2 mg/kg (P < 0.02). The results show that irinotecan, doxorubicin HCl, paclitaxel and fluorouracil have demonstrated utility as controls for future xenograft studies.

**PS35 A Novel Rat Osteotomy Model for Investigations of Fracture Healing and Mechanical Signal Transduction**

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Approximately 1.5 million fragility fractures occur annually in the U.S., with direct cost estimates approaching 14 billion. Promotion of fracture repair through biologic or mechanical stimulation possesses exciting potential for the design of new treatment strategies, especially once the underlying signaling mechanisms regulating the healing process are better understood. Distraction osteogenesis has been the standard model for application of mechanical loads to fracture sites. While this mode of mechanical stimulation has resulted in the production of new bone, it does not enable complete control of the local mechanical strain environment. Thus, it is not possible to determine the precise mechanical forces that elicit bone cell biosynthetic activity. We have developed the first in vivo model that enables the study of how mechanical stimulation influences the cellular and molecular processes that regulate fracture healing under normal, aged and osteopenic conditions. The model uses either young adult, ovariectomized, or aged Fischer 344 rats that receive an open, 0.6 mm femoral osteotomy under general anesthesia. The fracture is stabilized with a custom four-pin external fixator that enables normal behavioral and postural activities postoperatively. The temporal sequence of wound healing within the osteotomy, which recapitulates an endochondral repair process, has been demonstrated histologically. The unique feature of the model is that the fixator can be attached to a loading device that is calibrated to apply a controlled axial, compressive load to the tissue with the fracture gap. Essentially, the device applies cyclic pressure from a proximal
to distal direction across the osteotomy, which results in recoverable displacements of the tissue, similar to the squeezing and releasing of a sponge. Animals may be anesthetized for a series of consecutive daily loading sessions, or they may receive a single load stimulus, after which the gap tissue is harvested at a series of postload timepoints. The harvested tissue is snap-frozen for the planning of the installation should not be underestimated. The work practices and logistics of the building have to be work effectively. The impact of logistics and work practices on productivity demands, space available in the cage wash area and additional requirements of the expansion.

We are expanding our housing and testing facilities at The Neuroscience Research Centre. We have had to look carefully at how our support services supply clean cages and bottles to the various animal areas.

Our decision to use flexible automation was based on the consideration of ergonomics, laboratory animal allergens, productivity demands, space available in the cage wash area and additional requirements of the expansion.

The work practices and logistics of the building have to be completely integrated with the robotic systems so that they will work effectively. The impact of logistics and work practices on the planning of the installation should not be underestimated. This presentation will review how the operation of a robotic bottle processing system (that removes bottle caps, empties bottles, washes caps and bottles, thermal-disinfects, fills bottles and places caps back on bottles) and a robotic cage wash system (that empties cages, washes cages and places clean bedding in cages) have impacted on sterilizing, palletizing, assembly of cage components, movement, handling and storage in our animal units.

BP Benavides1,*, MF Starost2, M Flores1, IB Gimenez-Conti1, J-L Guénet1, CJ Conti2

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We have previously described an autosomal recessive mutation named nakt (nkt) exhibiting partial alopecia with CD4+ T-cell deficiency. Recently, we reported that nkt (now Ctslnkt) comprises a deletion in the cathepsin L (Ctsl) gene. Original efforts using nkt mice have been concentrated on the immunological phenotype; the current study focuses on the dermatological aspects of the mutation. For studies of hair follicle (HF) morphogenesis and cycling, groups of three male and three female at 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30 and 180 days postpartum from nkt/nkt and +/nkt littermates (BALB/c and C57BL/6 backgrounds) were used. Careful histological analysis of skin development of nkt mice revealed a delayed HF morphogenesis and late onset of the first catagen stage. The skin of Ctslnkt/nkt and Ctslnkt/nkt mice showed hyperplasia of the sebaceous glands along with structural alterations and abnormal distribution of HFs. Focal areas of keratin 6 expression in the interfollicular epidermis were associated with mild hyperproliferative skin in the mutant mice, suggesting that the normal biology of keratinocytes is altered. Also, we observed overexpression of profilaggrin/filaggrin in the epidermis of nkt mice, suggesting the involvement of Ctsl in the processing of this protein. Severe epidermal hyperplasia, acanthosis, and hyperkeratosis were only observed in mice maintained in contaminated environments. The analysis of BALB/c-Rag2-/-/Ctslnkt mice (skin collection at 2, 3 and 6 weeks of age from three males and three females) indicates that the skin defect remains under the absence of T and B cells. We describe for the first time the pathology of the nkt skin and provide further evidence in vivo that the lysosomal cysteine protease Ctsl plays a critical role in HF morphogenesis and cycling as well as epidermal differentiation. This animal model provides a tool for understanding the role of Ctsl in normal epidermal function.

PS38 The Integration of Flexible Automation in a Laboratory Animal Facility

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PS39 The Efficacy of Vapor Phase Hydrogen Peroxide against Nematode Infestation: The Caenohabditis elegans Model

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The group nematoda consists of approximately 20,000 described species, with actual estimates of 40,000 to 1 million
species in existence. The taxonomic groups of Rhabditida, Ascaridida, Oxyurida, and Spirurida pose parasitic health problems to humans and other vertebrates. Parasitic nematode contamination of Laboratory Animal Research (LAR) facilities can cause severe material, time, and financial loss on an annual basis. Entire rodent colonies may be infected in a short period of time, causing disruption of ongoing research and development. Of particular concern is the environmental persistence of nematode eggs. Current methods of decontamination of infested facilities, such as sodium hypochlorite, alcohol, and formaldehyde, are not effective, very labor-intensive and may pose substantial health and safety concerns. Vapor Phase Hydrogen Peroxide (VPHP) technology offers a useful and effective alternative to traditional methods of decontamination. VPHP Decontamination Systems are mobile or modular units that deliver and control VPHP into an enclosed chamber or room. The applications are typically closed-loop, with an inlet and outlet return from the enclosure, and have been widely used for area decontamination. To determine the efficacy of this application against nematodes, Caenorhabditis elegans, from the taxonomic group Rhabditida, was used as a model. C. elegans has proven to be a useful model for this type of application because it is nonparasitic to humans, rodents, and other vertebrates, but demonstrates extreme resistance to oxidation. Genome mapping on C. elegans was recently completed and more than 40% of parasitic nematode genes exhibit high levels of homology to C. elegans. This includes typical nematode parasites found in LAR facilities such as Syphacia muris, Syphacia obvelata, and Aspicularis tetraiptera. Processes to isolate eggs from adult cultures include exposure to 0.5-1% bleach for several minutes. The eggs remain viable despite this treatment. Unlike vertebrate parasitic nematodes, C. elegans does not need an animal host for activation and can be grown in vitro on culture plates. The animal model in the form of all life stages was placed on media growth plates then exposed grown in vitro.

**PS41 Analysis of the Efficiency of Single-Sided Versus Double-Sided Work Stations for Changing Mouse Cages**

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The growing trend for maintenance of extensive breeding colonies of genetically altered mice coupled with an increase in investigator exchange of mice has resulted in increased concern regarding the microbiological status of animals. Biosecurity concerns have prompted a transition from use of open-topped, conventional cages to microisolator cages, with manipulation of the animals performed in laminar flow, HEPA-filtered work stations. Cost of animal care, including labor cost, has become a rate-limiting factor for many institutions. This experiment focuses on analysis of the labor investment associated with use of single-sided versus double-sided (pass-through) HEPA-filtered laminar flow work stations. Individual technicians worked on single-sided work stations and two technicians worked on double-sided work stations to perform complete change-outs of static microisolator mouse cages. Work stations were positioned 7 ft from the animal housing rack. Carts for clean cages, water bottles and dirty cages were placed in standardized locations. The time to change one side of a rack was recorded for each of three experienced technicians (A, B, and C) and three two-person teams (A + B, A + C, and B + C), in four replicates. Mean times (seconds/cage ± SEM, n = 4) to change the cages on a static rack (average 34 cages) for individuals and teams were 53.7 ± 0.80 and 34.6 ± 0.56, respectively. This difference was significant using an unpaired t-test (P < 0.001). One way ANOVA with pairwise posttests of significance adjusted for multiple comparisons revealed no differences among individuals (P > 0.01), nor among teams (P > 0.01). All three teams were faster than individual A (P < 0.01). Teams A + B and B + C were faster than individual B (P < 0.01). There was no difference (P > 0.01) between individual C and any of the teams. These results demonstrate that on average, teams of two technicians changed cages faster than one technician. However, the mean time for two technicians to change cages was greater than half the time for one technician to change cages, indicating that it may be more efficient for animal care technicians to work individually.
The NHP Training Program was developed from existing training and proficiency assessment and staff progression. Observations: Although some programs have been developed to train both the novice technician without prior NHP experience as well as the senior, experienced technician interested in expanding their NHP skills. We will describe initial program implementation, including content, knowledge and proficiency assessment and staff progression. Observations: The NHP Training Program was developed from existing training initiatives—including applicable SOPs, guidelines, and training procedures—supplemented by new content and documentation. The program addresses both basic and advanced NHP husbandry and technical procedures. It provides comprehensive informational resources and appropriate guidelines for the orientation and training of all animal care personnel to the husbandry, safety, and technical skills necessary for working with NHPs. As designed, the program is arranged into three sequential tiers, with proficiency at one tier a prerequisite for advancement to the next. By using progression based upon demonstrated competence, staff may advance to higher levels over varying time periods based on knowledge and skills testing. All Animal Care Technicians (ACT) are expected to complete the entry-level tier (Tier I) and a pool of ACTs are expected to complete training at the more advanced levels, Tiers II and III. Conclusions: In summary, the NHP Training Program provides a formalized, tiered approach to the essential staff development needed for the care of NHPs. By establishing standards and documentation for staff knowledge and skills proficiency, the program greatly facilitates the management of NHP animal colonies while assuring high standards of care and regulatory compliance.

PS44 Proactive Compliance: A Team Program Approach to Revitalizing Primate Enrichment

KJ Hopper*
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The Division of Laboratory Animal Resources (DLAR) at the University of Pittsburgh proactively instituted a nonhuman enrichment plan that is founded on the USDA Final Report on the Environmental Enhancement to Promote the Psychological Well-being of Nonhuman Primates, July 1999 (The Final Report). This document is a draft policy of the USDA that has not yet been enacted. In anticipation of these standards becoming policy, the DLAR, IACUC, primate user groups and our enrichment specialist compared these new standards to our previous IACUC-approved plan. Our goal was to be “proactively compliant” to anticipated policy changes described in The Final Report. We established a program that was consistent with the five critical elements of The Final Report and our goal to have a revitalized enrichment plan that applied internal evaluation for continued improvement.

A task force was implemented to review current literature and regulations on enrichment. Then a subcommittee consisting of veterinarians, impacted investigators and IACUC members was formed. They established criteria for dispensation from plan elements, brought current protocols into compliance, shared enrichment and documentation techniques and considered research methods in decision-making.

In addition, a primate enrichment specialist position was developed and recruited. The enrichment specialist worked with investigators to evaluate enrichment and documentation needs, organized and implemented plan structure. The DLAR staff provided animal care and veterinary insight and reported to the IACUC. Investigators discussed how research and enrichment impacted each other. The IACUC considered these issues before passing the plan.

Our revitalized plan is running smoothly. The enrichment specialist oversees plan implementation and documentation. The DLAR assists enrichment and animal assessment. Investigators assist with assessment, provide enhanced enrichment and document their progress. The IACUC addresses dispensation requests through designated review on the large animal subcommittee.
Trainers administering a training program often hesitate to introduce unfamiliar methods into their teaching styles, especially those relying on new technologies such as e-learning. Trainers may prefer to use traditional presentation methods, such as books and slides, because they are accustomed to the use of these materials and can develop lessons with an economy of preparation time. Incorporating new technology into training programs upsets the balance by requiring the trainer to acquire and use new skills, which is a challenge made greater by the lack of time available to the trainer for such activities in the workday. As a result, the use of traditional teaching styles continues to restrict the learning process to the classroom environment where the trainer teaches through lecturing. This traditional approach has failed to facilitate learning for the nontraditional and independent student. The panelists will discuss adapting new technologies to a traditional training program through the use of the new AALAS LAT Companion CD, which is a compilation of study aids designed for accommodating a variety of adult learning styles and suitable for use by an individual, a small group, or a large class.

PS46 Conducting Workshops to Affect Student Attitudes towards Medical Research Using Animals

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Several public opinion polls have revealed a general lack of understanding of the biomedical research process. This permits groups who are opposed to animal-based research to influence public opinion towards medical research using animals. Since these groups have targeted the school-age public, the Student Science Literacy Workshop was designed to give high school students a better understanding of the research process. Through funding from the AALAS Foundation, academic institutions and pharmaceutical companies were encouraged to host a workshop, inviting approximately 30 students for a one-day visit. The workshop was a collaborative effort between the host institution and the Pennsylvania Society for Biomedical Research. During the visit, the students received an overview of biomedical research. Research scientists told students about their work, careers, and education. A laboratory animal veterinarian discussed the care and use of laboratory animals. The students toured the animal facilities and research laboratories. Several institutions required encouragement to accept this new paradigm of openness. After six workshops, it was evident that the students learned that laboratory animals are both well cared for and necessary for the advancement of medicine. However, the students said they were "bored" by the "lecture" type program, so we moved to a more interactive format. We developed guidelines encouraging presenters to show how their work is relevant to the students. We also developed a highly visual, interactive, game-type format as the basis for the program. The students enthusiastically received this revised format, and the positive messages concerning animal-based research and the favorable images of the institutions conducting this research were still strong. The Student Science Literacy Workshop demonstrated that allowing students to see the why and how of biomedical research increases their support for this research. Our long-term goal is to develop this program for nationwide distribution.
veloped to comply with and support new corporate-wide Time Out for Safety (TOFS) initiatives that placed responsibility on every staff member—from line management through husbandry and support staff—for analyses, implementation, training and periodic self-auditing of safety procedures in their areas. This presentation describes initial program development, implementation and safety outcomes. Observations: TOFS makes the fundamental assumption that all departmental staff must recognize and distinguish safe from unsafe work habits, conditions and procedures and take action to correct or report them. Steps in TOFS program development included setting goals and timelines; recruiting committees and task teams; expanding safety awareness and mandatory safety training programs; obtaining corporate support for incentives; developing a light duty work program; as well as creating procedures for action resolution, program monitoring and documentation. A formal program manual was developed and distributed to staff. Experience gained through implementation and critical staff input were used to better focus and manage the program. During the first year of implementation the TOFS Program contributed to 62% and 93% reductions in recordable accidents and lost time, respectively. Changes were noted in both work and home-related incidents. Key factors in the program’s success included senior management support, multi-modal safety awareness with employee involvement and incentives for goals met at individual and team levels. Conclusions: A coordinated program that enlists staff participation can markedly improve employee safety and well-being.

PS49 Unilateral 6-hydroxydopamine Lesions of the Nigrostriatal Dopamine System as an Animal Model of Parkinson’s Disease

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Parkinson’s disease is a progressive neurodegenerative disorder, the prominent neuropathological characteristic of which is the loss of dopamine (DA) neurons in the nigrostriatal system. There are several animal models to study different aspects of this disease; one of the best known is the 6-hydroxydopamine (6-OHDA) unilateral model. In this model, the neurotoxin 6-OHDA is injected unilaterally into the nigrostriatal DA system, which leads to a depletion of this neurotransmitter. Such a unilateral lesion leads to a number of lateralized deficits, especially spontaneous turning to the side of the lesion (ipsilateral), and contralateral sensory neglect. Other behaviors like grooming, locomotion and rearing can also be affected. The degree of DA depletion reached determines the extent and time course of these deficits. This model, as other models, is a very good tool to understand the pathology, the underlying compensatory processes, and perhaps someday to find a better therapeutic approach for the Parkinson’s disease.

PS50 The Rat as an Immunological Tool: No Longer Just for Mice

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Traditionally, rats have been heavily used as in vivo models in the fields of behavior, physiology, toxicology, tumor biology and transplantation. However, a review of the literature today references the rat as the second most frequently used animal in immunological research after the mouse. For example, a large number of genetically well-defined inbred laboratory rats strains are now available and a variety of congenic, mutant and recombinant rat strains of immunological interest have been developed in recent years. Also, a number of reagents such as monoclonal antibodies to rat cell surface antigens, soluble factors, cytokines and chemokines are now available. Finally, there are a number of rat autoimmune disease models that develop spontaneously after immunization with antigens, chemical treatments or upon introduction of a transgene. Data will be presented that summarizes representative examples of the points mentioned above; including in-house analysis of some widely used immunological rat models. Undoubtedly, experimentation in rats will continue to play a critical role in biomedical research, and rodent users need to be well-informed of specific reagents available today for this animal and the opportunities it has to offer as a valuable tool in the field of immunology.

PS51 Innate Response Induction in SCID Mice

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C.B-17/Uni SCID mice have a recessive mutation (SCID) that was identified by Bosma on chromosome 16. The animals don’t have normal T and B cells, due to the deficient activity of VDJ recombinase. Since its description, the SCID mouse became a valuable model for cellular and humoral immunology research. The innate response in vivo against Trypanosoma cruzi was studied, comparatively with BALB/c/Uni and C.B-17Scid/scid/Uni infected mice. The animals received an intraperitoneal infection with 10^6 trypomastigotes from Trypanosoma cruzi (TC)/animal, and evaluated the parasitemy and mortality. The C.B-17Scid/scid/Uni strain presented 100% of mortality, comparing with BALB/c/Uni mice, which controlled the parasitemy with a survival tax. In relation the in vivo experiments, C.B-17Scid/scid/Uni normal spleen cells were cultivated with Trypanosoma cruzi alive forms derivated from cellular culture, using different parasite concentrations. The cell activation results represented as cell proliferation-rates, showed that, in spite of cell/parasite relationship, the TC was able to induct cell proliferation and IFN-γ production. During the infection, when stimulated with TC, the infected mice cells showed a gradual loss of proliferation capacity, in both strains. The IFN-γ dosage started increasing by the 7th day of infection, with a peak in the 15th day of infection, and a control in the 18th after infection. The NO dosage in both strains didn’t show difference in the secretion levels among TC stimulated cells in vivo. The results show that SCID mice are able to induct NO and IFN-γ production, with a similar kinetic of the immunodeficient animals. In another experiment at our laboratory, it was possible to show how repopulate SCID mice with lymphocytes T become resistance against Trypanosoma cruzi. Both subpopulations CD4+ and CD8+ T cells showed capacity on intracellular parasite regulation. Our results suggest that the initial stimulation of CD4+ T-cell induced the toxicity function of CD8+ T cell.

PS52 Evaluation of Experimental Infections for Further Clinical Trials in Canine Visceral Leishmaniasis

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Visceral leishmaniasis (VL) is a zoonotic disease transmitted by phlebotomine vectors, in which the dog is the principal domestic reservoir. It has been postulated that dog vaccination could be the most efficacious control method, and therefore the establishment
of infection protocols is key to clinical trials of vaccine candidates. In this study, 21 female dogs, 4-6 months of age, were inoculated with metacyclic promastigotes of Leishmania chagasi harvested from colonized Lutzomyia longipalpis that were artificially infected. Four different schemes were used: 1) 10⁴ parasites i.v.; 2) 10⁴ parasites i.d.; 3) 10⁵ parasites i.v.; and 4) 10⁴ parasites i.d. Seven uninfected were used as controls. Animals were evaluated clinically every 15 days, and both clinically and parasitologically every 2 months. At 6 months p.i., the clinical evolution was variable within each group. Dogs subjected to the high i.v. scheme (10⁴) progressed rapidly towards overt disease, but i.d. infections also produced a variable degree of signs and symptoms (lymphadenophathy, onicogryphosis, dermatitis and chauquexia), which interestingly were more frequent with the low inoculum (10⁴). Intravenous inoculations lead to a high frequency of lymph node infection (50-66%), and infectivity to L. longipalpis (2/10 dogs), as opposed to the intradermal schemes in which lymph node involvement was low (20-33%) and no infectivity to vector could be detected. Also, intravenously infected dogs showed the highest antibody production (ELISA, mean O.D. = 1.06) and cellular immune response (2.79 stimulation index), associated with the lowest hematocrit values (33%). These preliminary results suggested that for short-term studies (<6 months) in which clinical symptoms and infectivity to vectors are used as endpoints, only intravenous infections with 10⁴ or 10⁵ metacyclic promastigotes could be used. The intradermal route possibly mimics the natural, protracted evolution of canine VL.

**PS53 Consequences of the Contamination of Ocular Lesions with Pasteurella pneumotropica in Nude Mice**

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Eleven nude (N:NIH (S)-nu) mice were sent to our institute due to the presence of subcutaneous abscess in the periorbital zone, which in most of the cases produced serious pressure on the ocular globe. In order to identify the cause of the observed lesion, the animals were euthanized under inhaled anesthesia. A complete necropsy was performed, but no lesions were found on the other organs. The abscesses were surgically removed and morphologically described; small cystic foci filled with a creamy content were present. Samples of this exudate were Gram-stained and cultivated on blood agar, cetrimide agar and McConkey agar. Histopathological diagnosis was also performed. Microbiological test showed the presence of Pasteurella pneumotropica, which was confirmed by microagglutination techniques. The present paper reports the presence of Pasteurella pneumotropica as a contaminant agent of ocular lesions. It is important to highlight that care must be taken on the procedures and maintenance of immunosuppressed animals, creating sanitary barriers to avoid the introduction of micro-organisms into the colonies.

**PS54 Antitumor Properties of an Anti-Idiotypic Monoclonal Antibody in a C57BL/6 Allogenic Mouse Model**

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We elucidated the antitumor effects of 1E10 (Ab2), an anti-idiotypic IgG obtained from a IgM monoclonal antibody named P3 that reacts specifically with N-Glycolyl containing gangliosides and also recognizes antigens in human breast and melanoma tumors. A murine tumor cell line positive for the P3 antibody (B16 Melanoma) in C57BL/6 mice was employed. The antitumor effect of 1E10 Mab was demonstrated in assays evaluating experimental metastases in allogenic animals, where mice received with a single dose low dose of Ab2 Mab alone i.v. 10 days after tumor cells were injected. In those experiments, a significant reduction of number of B16 melanoma metastatic lung nodules was observed in comparison with those from mice treated with an irrelevant Mab or with PBS. The mechanism through which 1E10 exerts its antitumor effects is still unknown, but it is clear that 1E10 Mab antibody could activate an antitumor response in metastatic progression.

**PS55 Experience in Surgical Experimental Rats and Hormone Replacement Therapy**

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We prepared different experimental rat models to study the effects of endocrine glands on the cardiovascular system. The conditions for the survival of the animals and for hormone replacement therapy were tested. The experimental models were 1) adrenalectomized male rats with or without steroid hormone replacement therapy; 2) ovariectomized rats with or without estrogen replacement therapy; and 3) Goldblatt 1 kidney one clip rats (1K1C) and Goldblatt 2 kidneys one clip (2K1C). These last two models were used to obtain hypertensive animals. Surgical procedure was carried out under ether anesthesia and Ag-clips of 0.22 mm were inserted in the left renal artery. Failure to properly insert Agring or sham-operated animals were used as control. Blood pressure (BP) of rats was controlled in the animal room, and after three measurements (different days) of high BP the rats were transported to the laboratory for experiments. 1K1C rats developed significant increments in BP after 10 days, whereas 15-20 days were necessary to induce a rise in BP of 2K1C rats. However, the long-term survival of the 2K1C model was better than 1K1C. Adrenalectomized animals were maintained with 0.9% NaCl in the drinking water (survival 10-12 days) or received hormone replacement; 0.5 mg deoxycorticosterone or 5 μg/100g body weight dexamethasone by daily intramuscular oil injections. It was observed an aggressive behavior in the rats receiving hormone replacement after the second oil injection; therefore light anesthesia was established as a routine procedure before intramuscular hormone administration. Adult female ovariectomized rats or sham-operated animals were studied. Hormone replacement was done under different conditions: silastic subcutaneous implantation of estradiol, or different hormone doses (intramuscular injection). Laboratory studies indicate that 20μg/100g body weight every other day for 30 days gave the best results in cardiovascular studies. In summary, we developed different experimental rat models to study the effect of hormones in physiological studies.

**PS56 Comparative Study about Bioavailability of Iron from Infant Foods Using an Animal Model**

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The objective of the study was to determine the amount of iron, provided by an infant food, is available for use from the human or animal organism.
The study used the Depletion-Repletion method in 144 male Sprague Dawley rats. During the depletion phase, rats were fed with a diet free of iron. Four weeks later, we examined the hemoglobin levels in eight rats in order to estimate the depletion level (Hb < 6 g/dL through the Hemocue technique). During the repletion phase, which was repeated three times, rats were classified in six groups of eight, and were fed ad libitum with diets containing 0, 6, 12 and 24 mg Fe/kg diet. Ferrous Sulfate was used as standard. Two weeks later we verified the hemoglobin levels.

At the same time the bioavailability of iron from fortified infant foods with 6 and 10 mg from iron Ferrous Fumarate per serving of 90 g was determined. First group: 5.48 ± 0.84 g Hb/dL with diet casein was free of iron; second group: 7.66 ± 0.84 g Hb/dL; third group: 9.97 ± 1.41 g Hb/dL; and fourth group: 12.61 ± 0.81 g Hb/dL. The results using infants fortified with iron were: 14.14 ± 1.0 g Hb/dL with 6 mg of Fe and 13.87 ± 0.81 g Hb/dL with 10 mg of Fe.

Conclusion: Hemoglobin levels were repleted in direct proportion to the increases of iron at the end of the experimental period. There is not a significant difference (P < 0.05), between the values of hemoglobin, when rats were fed with fortified infant foods containing 6 mg and 10 mg of iron as Ferrous Fumarate at 90 g/serving.

PS57 Effect of Vegetable Protein (Soybean) Versus Animal Protein (Casein) on the Progression of Renal Damage and Its Role in RAS in the Experimental Model of Remnant Kidney (Nx 5/6)

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Introduction: The experimental model of remnant kidney (Nx 5/6) resembles the progressive chronic renal disease in the human characterized by proteinuria, hypertension and glomerular sclerosis. In different renal pathologies it has been observed that a diet with soybean protein improves proteinuria and the lipid profile, and slows down the histological renal damage. Changes in the animal protein concentration (casein) generate alterations in the components of the renin-angiotensin system.

Objective: To evaluate the effect of a vegetable protein (soybean) diet versus an animal protein (casein) diet at different concentrations on the progression of renal damage and its role with the renin-angiotensin system in the experimental model of remnant kidney (Nx5/6).

Methodology: We studied 40 male Wistar rats (280-300g) with Nx 5/6, divided in four groups of 10 rats each. Group 1 was fed a diet containing 6% casein; Group 2 was fed 20% casein; Group 3 was fed with 6% soybean; and Group 4 was fed with 20% soybean. All groups were maintained during 22 weeks; urine and serum were collected at weeks 2, 7, 12, 17 and 22 for several biochemical determinations. The animals were sacrificed at week 22 to obtain plasma and renal tissue for evaluation of RAS and histology.

Results: Significant differences in proteinuria were observed: Group 4 (303 ± 72 mg/day) versus Group 2 (383 ± 87; P < 0.05) from week 12 to week 22 (182 ± 62 mg/day versus 275 ± 21.2 mg/day; P < 0.01); serum creatinine at week 12, Group 4 compared to group 2 (1.09 ± 0.27 mg/dL versus 1.8 ± 0.88 mg/dL; P < 0.01) and at week 17 (1.21 ± 0.25 mg/dL versus 2.2 ± 0.86 mg/dL; P < 0.01); urea nitrogen Group 4 versus Group 2 from week 7 (47 ± 15 mg/dL versus 87 ± 44 mg/dL; P < 0.001). The groups with casein developed higher seric triglycerides and cholesterol levels. The groups fed a restricted diet (1 and 3) developed lesser proteinuria and azoemia levels than the groups fed a normal diet (2 and 4).

Conclusions: The rats fed with vegetable protein (20% soybean) showed a smaller alteration of the function and minor renal structural damage, as well as a better lipidic profile versus the group with animal protein (20% casein).

Posters

P01 The Teaching of a New Alternative Method of Rabbit Intubation to Students

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The rabbit is a useful biomedical research model as well as an excellent household pet. In the spirit of educational excellence, Becker College sought to add rabbit intubation into the curricula of the veterinary technology and veterinary science degree program. Veterinary technology students were asked to participate in an extension laboratory of their veterinary surgery course. The students who participated had not intubated a rabbit previously to the laboratory. The students were randomly assigned into one of two groups. The first group of students was taught how to intubate the rabbit by the classical ‘blind’ method. This method entailed holding the rabbit by its head while the intubator listened to the animal’s breathing through a partially passed endotracheal tube inserted through rabbit’s oral cavity. When the rabbit inhaled, the intubator would then introduce the endotracheal tube to the lower respiratory tract. This method has a long learning curve and the rabbit may endure trauma if intubation is not immediately successful. Repeated attempts can cause potentially fatal laryngeal swelling and edema. Alternatively, a second technique was taught to other students based upon the blind technique used to endotracheally intubate horses. This technique uses visualization (rather than hearing) to enable the student to pass a transparent silicon endotracheal tube into the trachea of the rabbit. With the rabbit in lateral recumbency, the head is maximally dorso-flexed and a clear endotracheal tube is passed into the pharynx. The endotracheal tube is observed for condensation as an indicator of correct position at the larynx. Continued observance of condensation indicates successful entry through the larynx. Students of the classical technique took longer to learn and achieve successful intubation. This new lateral intubation technique allowed students to successfully intubate quicker and decreased the risk of laryngeal swelling and trauma.

P02 A Transgenic SOP Companion for Animal Room Use

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There has been a tremendous amount of interest in developing criteria to help animal care technicians establish whether the
transgenic mice under their care are exhibiting normal behavior or are ill and require veterinary care. Many institutions have extensive standard operating procedures (SOPs) that cover all aspects of transgenic mouse care and health concerns. A common problem is that this information is usually not available in a short, clear, concise and moisture-proof format for use in the animal room. The needed information, if available, is almost always found in the official SOP book format along with all the other SOPs relevant to the animal facility. This book is frequently placed in an office or other general area that can be somewhat distant from the actual animal room. Most animal room technicians will not take the time to go and look up the pertinent SOP(s) to see if the observed mouse behavior is normal or abnormal for that particular transgenic strain. Because the information is not close at hand, this may result in large numbers of requests for the veterinarian or animal health technician to look at and evaluate what usually turns out to be normal behavior for that particular transgenic strain. The time wasted by the medical staff assessing healthy animals takes its toll on individuals already pressed for time. The Transgenic SOP Companion consists of a phenotypic description of each strain. It includes a photograph or photographs of the mouse, coat and skin color/patterns descriptions, eye color, general appearance, physical characteristics, behavioral characteristics and breeding characteristics. This presentation will describe the developmental process used to create the Transgenic SOP Companion, which is used for quick reference in the individual transgenic mouse room.

P03 An Effective Component of the Outreach Program: “Take Our Children to Work” Day

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Pharmacia—Kalamazoo has embraced “Take Our Children to Work” Day as part of its outreach program since 1999. Our management and employees believe the best public relations tool a company has to offer is the testimony from employees who care about and know the value provided by producing the best and safest medicines for families and pets like our own. Many talented people team up to deliver a well-rounded and fun-filled tour of different departments within our research and development facilities to children of company employees. We demonstrate procedures including animal husbandry and care, discuss safety issues, and describe other aspects of our daily routines. Contests are held and prizes awarded for some events and all children receive a variety of gifts to serve as a remembrance of this special day. The goodwill generated from this annual event is evident from the overwhelmingly positive and supportive feedback received from parents and children alike. This event also has the side benefit of serving as a means to educate lay employees about the importance of animal research, and allows our scientists and technical staff to demonstrate the respect, care and concern they have for the animals with which they work.

P04 Behavior Management and Records: Assessing, Addressing, and Tracking the Psychological Well-Being of Primates

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The regulations stemming from the Animal Welfare Act (9 CFR 3.81) list primates in psychological distress as one of the categories of animals requiring “special attention.” Primate laboratories everywhere have attempted to address in environmental enhancement plans this requirement and its implications, including how to assess and document the presence and treatment of psychological distress. However, very few comprehensive plans for the management of primate psychological well-being have been presented in a public forum. The NIH Veterinary Resources Program (VRP) behavior staff has developed a strategy for the management of psychological well-being, which includes a tracking and documentation database created using Microsoft Access. The results of a focal animal behavior observation are recorded in the medical record of each VRP housed primate twice yearly, as well as upon each animal entry into a VRP facility. The behavior staff also monitors each VRP housed primate weekly for any dramatic change in behavior. Any primate displaying an abnormal behavior during a semi-annual or facility entry observation or a significant increase in abnormal behavior during a weekly observation receives a treatment evaluation. During this evaluation, an attempt is made to determine the possible causes of the abnormal behavior and potential methods of treatment. Any primate placed on treatment for an abnormal behavior receives additional evaluations monthly, during which the effectiveness of the current treatment strategy and the possible need for a different approach is determined. Semi-annual and facility entry observations, notes from weekly observations, and treatment evaluations are all entered into a behavior tracking database, which produces labels that are placed in individual animals’ medical records. The tracking database and record documentation have helped shape strategies for the management of behavior for nonhuman primates housed in VRP facilities.

P05 Continuing Education Topics Presented by Staff Trainers at Bi-weekly Husbandry Team Meetings

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At our institution, two experienced animal care technicians hold dedicated positions as “staff trainers.” Their main responsibilities have been to provide training to new employees. However, it was recognized that staff training also needed to be an ongoing process, since seasoned employees are given new job schedules that might include species or procedures that would be new for that employee. Re-training of seasoned employees presents different challenges in comparison to training new employees, as seasoned employees may feel that they have completed any necessary training, and they may also fear embarrassment if they were performing a designated task incorrectly. It was determined that the staff trainers would give continuing education training sessions during the already existing bi-weekly team meetings. In that way, the trainers would visit with the employees at a time already preset in their schedules as opposed to the employees coming to the trainers and taking time out of their extremely busy workdays. It was felt that the trainers could adequately present the training topics and still not exceed the scheduled meeting length. This would allow all employees to experience a different continuing education topic every two weeks. The training sessions usually last between 20-30 min. The presentations of the topics are given in “hands-on” lecture or quiz formats, and are designed to be interactive and entertaining. Some examples of topics include a hands-on guinea pig and rabbit training session, a customer service skit, an AAALAC mock inspection, an animal research-themed “Jeopardy!” game, and a husbandry-themed Pictionary game. By attending all of the team meetings, the trainers gain firsthand
knowledge of the problems and/or issues the teams may be having, and they can use this knowledge to develop future training topics. The staff trainers have received positive feedback from the seasoned staff regarding both the topics and the format.

**P06 Development, Maintenance, and Use of an Animal Import Database: A Valuable Tool for Risk Management**

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Animals imported from outside sources present a risk of introducing pathogens into rodent colonies. Maintaining closed colonies in a dynamic research environment is difficult. Therefore, an import database was developed at our institution to facilitate evaluation and tracking of barrier health reports. The database was designed with two tables. In the first table, barrier health information was entered from the health reports that were attached to the animal shipments. In the second table, information regarding the animal order, including the barrier from which the animal was shipped, was entered. Using the barrier identification as the link between the two tables, a query was developed that pulled the order information, matching it with the barrier health report. By using the query to link the information by barrier, the health information for that barrier was entered once and updated only as new health reports became available, usually quarterly. Orders were entered weekly into the order table. A report was designed to output the health information for e-mail to investigators. The clinical veterinarian completed a weekly import report. The import database has been maintained since April 2001. No viruses were reported on the vendor health reports or for our outsourced colonies during this time period. The most commonly reported bacterial organisms on the vendor health reports include *Pasteurella pneumotropica*, *β-hemolytic streptococci*, *Klebsiella oxytoca*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Pneumocystis carinii*. Protozoa, such as *Entamoeba* spp., *Spironucleus* (Hexamasti) spp., and *Trichomonad* spp., were also reported. In addition to these organisms, the outsourced colonies reported *Helicobacter hepaticus*, *Helicobacter bilis*, and *Helicobacter* spp. Entering the health data into a database has allowed effective and efficient use of this information. This database has proved to be a valuable tool in managing the risk associated from importing animals into existing colonies.

**P07 DoseWatch: A Unique Tool for Monitoring Real-Time Dose Activities in a Toxicology Laboratory**

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Monitoring technician dosing rates (animals/minute) and the adherence to animal identification SOPs is necessary to ensure accurate dose administration. Dosing production rates vary by individual based on experience and the complexity of the dose. In addition, the utilization of transponder chips to positively verify animal identification can be overridden by the technician in the event of transponder failure. MPI Research has developed DoseWatch, a Microsoft Access-based application, to monitor these activities as recorded onto our automated data collection system in real time. Displayed at the supervisor’s desk are the technician ID, study room assignment, number of animals dosed each minute, if the transponder ID has been overridden, cumulative number of animals processed per dose session and data collection computer idle time. DoseWatch generates reports on these as well as the total amount of test material administered and if any animal received a duplicate dose. While there is no substitute for a personal visit to the animal room to observe the technical activities, with the aid of DoseWatch the study supervisors are better able to monitor 14,000 daily animal doses.

**P08 Managing Animal Concern Correspondence in a Web-Based World**

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The World Wide Web has provided animal rights groups with an effective and expedient way to spread their message and recruit supporters from every corner of the globe. Even the novice web-surfer can find samples of letters and e-mails, home and business addresses of researchers, and even directions on how to conduct terrorist acts without being caught. Now, an animal rights enthusiast can simply copy and paste a posted animal rights message, and bombard the targeted organization with e-mails and letters. Handling the resulting influx of correspondence can be a daunting task for animal research organizations. Returning a point-by-point response to each communication may fuel follow-up correspondence that can drag the institution deeper into a never-ending debate. Not responding at all might give the impression that the institution either does not care or has something to hide. Laboratory animal care and research personnel often feel like they are stuck between the proverbial rock and hard place. A well-considered plan of action can help to evaluate and respond to animal concern correspondence in an appropriate and timely manner. The formulation of a correspondence management strategy should include input from a variety of institutional representatives, such as researchers, research administrators, public information officers, security professionals, attending veterinarians, animal facility managers, and members of the IACUC. At our institution, such a team was assembled and developed the following strategy for management of web-initiated animal concern correspondence:

- Monitor the Internet for information posted about research involving animals at the institution.
- Alert targeted personnel and key institutional officials.
- Prepare position or policy statements on anticipated animal concern topics in advance, and post them to the institutional web site.
- Develop criteria for determining when an animal concern communication merits a more individualized response.

This strategy has enabled our institution to keep abreast of current animal rights correspondence campaigns, address public concerns about animal research in an open and straightforward manner, and express our institutional pride in the quality of our animal research program.

**P09 Novel Training Concepts and Techniques Used to Increase Safety Awareness in the Workplace**

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Safety plays a significant role in managing research facilities. Safety training is a mandatory requirement of the Occupational
Health and Safety Administration (OSHA), and it helps to maintain a safe work environment for all employees. Most often the content and presentation of the required safety training is repetitive from year to year. This naturally causes a decline in the employees’ interest levels, which consequently limits the amount of important information that is learned from the training. Creative and innovative safety training techniques can be used as novel tools to increase safety awareness in the workplace. Novel concepts and techniques (i.e., interactive lectures, games, and musical presentations) have been incorporated into the safety training program in the Department of Laboratory Animal Resources. Following implementation of the training techniques, the LAR division of the Safety Assessment facility experienced a ~40% decrease in the number of workplace injuries from 1999 to 2000 and an additional ~31% decrease from 2000 to 2001. Additionally, an employee opinion safety survey indicated that ~88% of employees surveyed felt that safety training incorporating the interactive and novel techniques/concepts was the most effective type of training. Furthermore, ~96% indicated that the monthly training sessions were motivational and educational. In conclusion, using innovative training techniques is an integral part of the learning process for many employees. When used as part of a safety program, it can help to make the information interesting and retainable, thus increasing safety awareness in the workplace.

P10 Palm OS Applications Facilitating Biomedical Research

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Palm OS-based (PalmPilot, Visor, etc.) devices facilitate the way we handle our schedules, contact information (e-mail address, telephone numbers, etc.), and other essential day-to-day activities. These devices support Macintosh-based or PC-based formats equally, plus through the infrared (IR) ports permit easy quick data transmission to other individuals with these devices, and printing to the multitude of IR port equipped printers.

Aside from the devices’ “out of the box” utility, the devices are programmable to facilitate the unique needs of investigators, veterinarians, facility managers, and an IACUC. The authors present additional utilities that have been created to facilitate process and increase communication between and among these components of biomedical research.

The additional utilities include automation and acceleration of animal facility-related problems (lights out, door fails to close, etc.); animal death notification; and IACUC inspection reporting. The electronic forms created require important information to be provided as the user steps through completing the electronic form. Once the user performs a “HotSync” operation at their computer, the information from the Palm device is entered into a SQL server or Access database. From here an automatic notification can be generated to the facility manager for facility problems and to the investigator for an animal death notice.

The use of the Palm for IACUC semiannual inspections can help ensure a more consistent inspection by stepping the user through a given series of questions. Using the Palm for IACUC inspections also provides the applicable regulations and IACUC guidelines in a searchable format in the Palm device.

P11 The SWAALAS Outreach Committee—Bringing People and Animals Together in the Community

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The Southern Wisconsin branch of AALAS founded an “Outreach Committee” in 1997. Then-SWAALAS President, Monique Heiser, had a vision of taking the people involved in animal research and their compassion for animals to make a difference in the community through various volunteer activities. The committee chose several activities to coordinate on a monthly and annual schedule and is in its sixth year of operation.

The committee coordinates the following activities:

- Pets and People—members meet once a month at two local nursing/retirement homes to bring their furry companions and pet-loving residents together. The reward of a resident’s smile and a pet’s returned affection are heartening.
- Ferret Nook Volunteers—members meet once to twice a month at a local non-profit ferret shelter to help with the administration of medicines and general housekeeping. This shelter relies on the help of the volunteers to maintain the health of 100+ ferrets.
- Annual Spring Canine Tattoo Clinic—experienced and qualified members provide permanent tattoos registered with the National Dog Registry applied free of charge. This service is possible with the help of the Madison Area Technical College—Laboratory Animal Technician program students and facility. Since 1998, over 100 dogs have been registered.
- Annual Pet Food and Supply Drive—each year, two local non-profit animal shelters are chosen to receive the benefits of a week-long collection.
- Girl Scout Pet Care Badge Clinic—members help local girl scout troops complete the requirements to earn a Pet Care Badge by conducting a clinic of specific activities. Six troops have earned badges.
- Middle School Presentations—started in 2000, members have made “Career Day” presentations for laboratory animal research.
- All SWAALAS members are encouraged to be part of an Outreach Committee activity and kept informed with newsletter articles and updates at the branch meetings.

P12 Use of PalmPilots and Animal Database in Studies Involving Long-Term Glucose Sensor Evaluation

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One of specific problems of glucose sensor studies is to accurately record time of each blood sample, obtain from the diabetic animal, in order to compare the results to the sensor calculated glucose. In the past, each technician used a clock and recorded animal, in order to compare the results to the sensor calculated glucose. In the past, each technician used a clock and recorded animal, in order to compare the results to the sensor calculated glucose. In the past, each technician used a clock and recorded animal, in order to compare the results to the sensor calculated glucose. In the past, each technician used a clock and recorded animal, in order to compare the results to the sensor calculated glucose. In the past, each technician used a clock and recorded animal, in order to compare the results to the sensor calculated glucose. In the past, each technician used a clock and recorded animal, in order to compare the results to the sensor calculated glucose. In the past, each technician used a clock and recorded animal, in order to compare the results to the sensor calculated glucose. In the past, each technician used a clock and recorded animal, in order to compare the results to the sensor calculated glucose.
ent, diabetic animal glucose level was an average of 91 mg/dl and appeared relatively close to the non-diabetic dog. The handheld device, equipped with two separate screens—one for sensor readings and another for an actual measured glucose—was extremely helpful for this type of studies.

The Animal Database is based on simple Microsoft Access tables and Satellite Forms. The database contains the full history of each animal, starting from the day of its arrival and ends with animal disposition. It also contains easy-to-use information about animal health, and includes treatment records, surgical records and general health history like weights, type of food used, daily appetite, etc. The main objective of the database is to maintain an accurate, easy-to-retrieve record of implanted sensors and also GLP and non-GLP study results.

The Animal Database also tracks and records supplies and inventories in a GLP-compliant manner. Every material, chemical, or drug entering the animal facility undergoes thorough inspection. If an inspected item is in compliance with all specifications and requirements and comes from the approved vendor, it receives a unique reference number and is released for the use in the lab. Otherwise, it is sent back to the vendor for credit or replacement.

The Animal Database also contains useful information about current SOPs, records animal studies, and contains safety data about chemicals used in the lab, including MSDS and California Prop 65 (materials that can cause cancer and reproductive harm). The database is also integrated with a Microsoft Outlook application that contains emergency phone numbers, business contacts and vendors’ contacts.

Having a large population of diabetic animals as well as different types of implanted sensors, it becomes extremely important to be able to sort and classify incoming study data from the very beginning—right from the moment when the data is collected. That is why using handheld devices is very useful. The main advantage of the created Animal Database is its design as an open system. It means that an investigator can easily add more tables of queries at any time, depending on the type of study run, or choose appropriately trained personnel to perform a certain study.

Palm OS-based devices are a very useful tool of collecting, sorting, analyzing and retrieving scientific information in the animal lab.

P14 Using a PDA to Computerize Rodent Cage Inventory to Facilitate the Ordering of New Caging in a Timely Manner; or “What Do You Mean, We’re Out of Mouse Cages to Change With? We Just Received a Shipment Last Month!”

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One of the many challenges faced by animal care facilities is how to balance the need for new rodent caging against tightened operating budgets. New caging is needed on a regular basis to accommodate growing populations of animals and to replace aged and damaged units. It is vital to have adequate caging supplies to meet user needs, but at the same time it is important to quantify the actual need so that too little or too much equipment isn’t purchased. At University Animal Care, the Cagewash and Purchasing Supervisors researched and established the “rules” needed to quantify the optimal ratio of rodent cages in use in the animal rooms to the number of cages needed for cage changing. A personal digital assistant (PDA) was purchased to gather inventory data of the caging available for cage changing. A simple spreadsheet was designed to compare the number of caging units in use in the animal rooms to the number available in the cagewash area. The spreadsheet calculates the amount of additional caging needed (if any) for each of the animal care facilities. Having hard data and quantifiable figures is a huge aid in justifying additional caging purchases to upper management. Using a set of standardized data has also allowed the department to budget for future rodent caging expenditures based on past trends. By using this system, University Animal Care, has successfully moved the process of purchasing additional rodent cages from one of “gut feeling” to a much more justifiable and scientific method, thereby increasing efficiency and fiscal responsibility.

P15 Creation of a Global Database on the Safe Use of Formulation Vehicles in Preclinical Studies

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In the course of drug discovery and development, many preclinical in vivo studies are conducted on new chemical entities (NCEs) to investigate and optimize the pharmacodynamic, pharmacokinetic, and safety characteristics of the drug candidate. A consistent challenge for investigators conducting these studies is overcoming unfavorable physiochemical properties of the NCE (e.g., poor solubility) to create a suitable formulation to deliver the desired quantity of the NCE to the animal model by the intended route of administration. Investigators use one or more relaxer chemicals known as excipients to create simple or complex mediums called vehicles in which solutions or suspensions of the NCE (formulation) can be created for dosing. An ideal vehicle is one in which the NCE can be dissolved or suspended to the desired extent, delivered by the intended route of administration for the desired duration, and has little or no appreciable pharmacologic or toxic effect. These latter properties allow for ethical treatment of animals but also ensure that properties of the NCE can be effectively deconvoluted from that of the vehicle. The pharmacologic and toxicologic effects of excipients and vehicles administered to animals is often nonexistent, anecdotal, difficult to find and collate, and complex. As a result of this and aggressive time lines, unprecedented, exotic, and uncharacterized excipients or vehicles whose effects are unknown is gaining wider prevalence in the industry, thereby potentially compromising, confounding, or invalidating important study results.

Given the critical nature and broad impact of vehicle use and safety in drug discovery and development, several departments at our company sponsored the formation of a Vehicles Safety Task Force (VSTF). To effectively address the proper use of vehicles and assist with the accurate interpretation of data from preclinical studies, the creation of a Vehicles Safety Database containing information on excipients, and vehicles was undertaken. This globally available, web-accessible, relational database system is used to collect, manage, categorize and report information on the pharmacologic and toxicologic effects of excipients and vehicles, as well as their overall properties and uses in drug discovery and development. The database assists researchers with the selection of the most appropriate vehicles for their application, provides an understanding of the effects of excipients and vehicles on preclinical species, and deconvolutes these effects from study results, thereby reducing timelines and achieving commercial advantage.
P16 Nonhuman Primate Enhancement and Positive Reinforcement Training Computer Database

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Record keeping is a critical component of a good research program, but maintaining records manually can be tedious and time-consuming. Many commercially available software programs provide more efficient record keeping functions, but they may be menu-driven, difficult to learn, have limited flexibility, and require time-consuming and expensive maintenance and technical support. We required a software program to develop an environmental enhancement and positive reinforcement-training database for nonhuman primates. We needed the program to help us collect, store, and display extensive amounts of data in various ways, and to be easy to use and maintain. FileMaker ProTM (FMP) 5.0.1, a user-friendly relational database software package, was employed. This application enabled sharing of files throughout our local area network. The database allowed us to establish an individual profile for each animal that includes experimental and medical histories, pair/social-housing information, and animal disposition. The database can be accessed from any networked computer and used to evaluate animals or provide new information to the files. The application enabled creation of reports for administrative or regulatory purposes by simple searching techniques. FMP allows assignment of passwords for access privileges to edit layouts and fields and perform selected activities. Access to specific information was limited to those who needed to view only, while other selected users were allowed to enter and manipulate the data. This suite of FMP files provided a secure database resource that is routinely maintained and backed up by our network. This reduced the paper trail and produced a streamlined process. FMP has increased the efficiency of and enhanced the data management in our computerized records system.

P17 Encouraging Enrichment with Visual Aids

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The fast-expanding research community and increased public interest in the biological research field has created a need to explain and demonstrate the continuing advances being made in environment enrichment for laboratory animals. We needed to quickly and consistently educate the rapidly growing and multicultural research staff, prospective employees, and the public about the behavioral enhancement devices available for use at our facility. For the wide range of species housed in our vivarium, we have found that a poster located just inside the front doors leading into the vivarium to be the most effective means of presenting this information. The poster contains pictures of the various species of animals housed in our vivarium interacting with the enrichment devices available for each species. The poster also contains a brief description of the enrichment device and how the animals may actively use the device. For example, one picture shows a swine searching for kibble in their clean bedding, while another shows dogs removing treats from inside a Kong toy. Visually informing new research staff, animal technicians and the public about the enrichment devices used and the reasons for their use seems to ease any apprehension or misconception previously perceived before entering the vivarium. It also helps the investigative staff make informed decisions about what enrichment devices they would like their research animals to have. This is especially helpful when describing the use of “mouse houses” and nestlets to improve breeding performance in mice. The poster is also one of the first things that people who are touring the facility encounter. They are usually unaware that enrichment devices are available and used by laboratory animals. Having seen the posters leaves everyone with a positive feeling that the research animals are well cared for and that we can provide an enriching environment for laboratory animals.

P18 Computed Radiography: Radiographic Processing Technique That Eliminates Costs Associated with Film, Reduces Retake Numbers, and Decreases Number of Radiation Exposures

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Computed radiography (CR) is a digital imaging technology introduced for use in human medicine in the 1980s by Fuji based on reusable storage phosphor imaging plates. The storage phosphor plates are similar to intensifying screens used in cassettes, except that when exposed to x-rays, intensifying screens emit light immediately. CR imaging plates respond to x-ray exposure by absorbing the radiation, and exciting the electrons into semi-stable states, storing the image temporarily. The latent image is read out by scanning the imaging plate with laser light, releasing the electrons from their trapped states. The scanning results in the plates giving off visible light, which is detected and converted to a digital image that can be displayed on a computer screen, archived as digital data in a searchable retrievable format, transferred to others by e-mail, or printed on photo-quality or plain paper. The phosphor imaging plate is then reset and available for the next radiation exposure. CR has been clinically validated in the human medical world for over 18 years, in a variety of applications. The need for retakes due to over and underexposure is reduced because CR has higher exposure latitude than film. Combined with image manipulation software that allows contrast and brightness adjustment as well as more sophisticated image processing algorithms, the user is able to view both soft tissue and bone detail from a single exposure, and optimize the image to maximize its diagnostic utility. The system eliminates the need for developing chemicals, expensive automated developers, and costs associated with film procurement or expired film, and can reduce total numbers of radiation exposures to people working in radiology. The cost of the equipment in the veterinary field is significantly reduced from the human technology, yet comparable in quality and readily available in the commercial marketplace.

P19 Strategies for a Successful Pre-Clinical Continuous Infusion Program in the Nonhuman Primate

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As the nonhuman primate (NHP) becomes increasingly important as an animal model for testing of investigational drugs, study designs that mimic clinical protocols become more complex. Intravascular administration is the most common infusion route. Successful infusion techniques require the integration of numerous technologies including catheter implantation, protecting externalized equipment and a method of compound delivery. Having the capability for both tethered and ambulatory models allows the researcher to select the model that is more appropriate for the physiochemical properties of the test article.
images were captured onto a PC computer file for data analysis. The IVC. Both lateral and AP images were taken to confirm DVT. ing contrast agent into the lateral tail vein, was performed using the IVC removed, and the animal closed. Venography, by inject-renal veins and all draining branches. Forty-eight hours rats weighing 250–350 g by ligating the vena cava caudal to the follow the resolution of a thrombus during treatment. for gross evaluation, there was no other non-invasive technology restored in the inferior vena cava (IVC) after thrombus forma-
mised by gas in the animal's bowels. Besides euthanizing animals resolution, but visualization of the thrombus can be compro-
tion. Ultrasound has been used to document thrombus 
mans than our previous ligation model because venous flow is 
care concern, with an incidence of approximately 250,000 cases 
therapeutic Cardiomyopathy Model

Objective: Deep venous thrombosis (DVT) is a national health care concern, with an incidence of approximately 250,000 cases per year. A non-occlusive animal model using contrast venogra-
ded to evaluate thrombus resolution. This model model more accurately represents the clinical etiology of DVT in hu-
mans than our previous ligation model because venous flow is restored in the inferior vena cava (IVC) after thrombus forma-
tion. Ultrasound has been used to document thrombus resolution, but visualization of the thrombus can be compro-
mised by gas in the animal’s bowels. Besides euthanizing animals for gross evaluation, there was no other non-invasive technology to follow the resolution of a thrombus during treatment.

Methods: A DVT was created in male Sprague Dawley (n=5) rats weighing 250–350 g by ligating the vena cava caudal to the renal veins and all draining branches. Forty-eight hours postligation the animal was re-opened, the main ligation around the IVC removed, and the animal closed. Venography, by inject-
ing contrast agent into the lateral tail vein, was performed using a fluoroscopy unit to estimate baseline thrombus mass within the IVC. Both lateral and AP images were taken to confirm DVT. Animals were treated after baseline venography. All venography images were captured onto a PC computer file for data analysis. Measurements in cm² were obtained, normalized to baseline thrombus area, expressed as a percentage of initial thrombus, and standardized to the vertebral length of each animal. Results: Animals treated with anti-inflammatory agents 1 and 2 showed greater thrombus resolution over 7 days posttherapy when compared to saline controls (0.34 ± 0.07, 0.34 ± 0.05 vs. 0.68 ± 0.13, cm² of thrombus, P<0.05). Conclusion: This model provides a non-invasive approach to visualize a formed thrombus in the IVC that enables investigators to treat experimental DVT and evaluate thrombus resolution over time.

P21 A Novel Tumor Model Derived from a Spontaneous Os-teosarcoma in VEGF-GFP Transgenic Mouse: Strong Expression of GFP in Tumor Cells
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Vascular endothelial growth factor (VEGF) mediates angiogenesis, tumor growth, and metastasis. Murine models of metastatic tumors with green fluorescent protein (GFP) expression driven by VEGF promoter can be imaged intravitally, as well as externally, may open many possibilities for real-time tumor angiogenesis, metastasis, and experimental treatment studies in vivo. A spontaneous tumor developed on the back of an 11-
month-old congenic VEGF-GFP/C3H female transgenic mouse in the breeding group of our defined flora animal colony. This transgenic strain was made on an FVB background and then backcrossed into a C3H background to make a congenic VEGF-
GFP/C3H transgenic strain. The number of backcrosses performed for this congenic strain was three at that time. Necropsy and histopathologic examination show a spontaneous osteosarcoma with distant lung metastases. Fresh tumor frag-
ments were successfully transplanted into homozygous VEGF-GFP/C3H transgenic mice and passedaged in vivo. In the first five generations, the tumor take rate was 100% (25/25), with a latent period of about 6 days, and reached an average tumor volume of 1500 mm³ at approximately 30 days. The trans-
planted tumors have maintained their original histopathologic characteristics and metastatic behavior. The tumor tissue also grows in wild type C3H/Sed mice with a 10/12 take rate, and grows as monolayer cells in vitro. Strong expression of GFP was found in all non-necrotic areas of the fresh tumor tissue, and more than 50% of cultured tumor cells in vitro when maintained as an exponentially growing monolayer. Real-time tumor growth was visualized by GFP fluorescence in tumors grown in transpar-
ent dorsal skin chambers of wild type C3H/Sed mice. This in vivo and in vitro transplantable and metastatic osteosarcoma exhibiting strong GFP expression in tumor cells is an attractive model for further study of tumor pathophysiology and treatment efficiency affecting VEGF expression. (This work was supported by NIH grants R24-CA-85140 and P01-CA-80124).

P22 Age-Related Cardiomyopathy in J2N-K Hamsters: A New Idiopathic Cardiomyopathy Model
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A J2N-K hamster, established by Saito et al. in 1999, is a new idiopathic cardiomyopathy strain. We describe the age-related cardiomyopathy in this strain of hamster. Eighty male hamsters were used. Five to 10 animals were sacrificed at the ages of 3, 4, 5, 6, 8, 10, 12, 16, 28 and 36 weeks, and their hearts were processed for light microscopy. Lactic dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine kinase (CK) in serum were analyzed at the ages of 3, 4, 6, 8, 12, 16 and 36 weeks.

At 4 weeks of age, small necrosis was identified in 1 out of 10 animals. In all animals of 5 weeks of age or more, myocardial abnormalities were present. At 5 weeks of age, multiple necrotic
fosical Rhesus Monkeys

In Vivo Sampling Laboratory, BAS Bioanalytical Systems Inc.,

5.0 french fenestrated silicone catheter that is placed 1 to 1.5 cm

key allows for direct access to the cisterna magna using a 3.5 or

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invasive trephination techniques to pass through the skull, in-

have proved successful and reliable but unfortunately require

mational, less-invasive method permits chronic, reliable collection

consistently collected CSF for over 6 months. This novel, eco-

We currently have instrumented animals from which we have

access for sampling CSF in a conscious, chaired rhesus monkey.

(VAP) placed subcutaneously between the scapulae to permit easy

sential behavior of the animal throughout the course of study.

foci and immature fibrosis were noted. In all animals of 6-12

weeks of age or more, necrotic and fibrotic foci, and calcifica-

were observed. In all animals at 16 weeks of age or more, the

most obvious changes in the heart were dilated ventricles and

atrial and /or ventricular thrombi. Increases in serum LDH, AST, ALT and CK were observed at 12 weeks of age or more, as

compared with younger animals.

This study suggests that the J2N-K hamster is a useful model

P23 An Alternative Method of Chronic CSF Collection in Con- 

scious Rhesus Monkeys

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Recent research priorities led us to develop an alternative

method of chronic cerebral spinal fluid (CSF) collection in con-

scious rhesus monkeys. Models have been established in the past

in nonhuman primates and canines, many of which implement

stainless steel cannulas into the lateral or fourth ventricles or

catheters into the cerebral subarachnoid space. These models

have proved successful and reliable but unfortunately require

invasive trephination techniques to pass through the skull, in-

volve the use of expensive and highly specialized stereotaxic

equipment for the precise placement of the implants, and may

result in exteriorized hardware that is cumbersome to maintain

and unaesthetic. The model we developed in the rhesus mon-

key allows for direct access to the cisterna magna using a 3.5 or

5.0 french fenestrated silicone catheter that is placed 1 to 1.5 cm

into the cisterna. The catheter is attached to a vascular access port

(VAP) placed subcutaneously between the scapulae to permit easy

access for sampling CSF in a conscious, chaired rhesus monkey.

We currently have instrumented animals from which we have

consistently collected CSF for over 6 months. This novel, eco-

omical, less-invasive method permits chronic, reliable collection

of CSF in conscious rhesus monkeys and has the additional ad-

vantages that the model is easier to maintain and more aesthetic.

P24 Automated Blood Sampling with Concurrent Electrocardio-

grams for Laboratory Animals

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Fully automated blood sampling for rats was first introduced

at the 1999 AALAS National Meeting in Indianapolis. Now that

this is an established technique, there has been an interest in

expanding the technology for use in other laboratory animals.

The work presented here outlines the progress that has been

made in using automated blood sampling for mice and dogs.

This study also reports expansion of the technique to include

concurrent recording of electrocardiograms for rodents and

dogs. The species studied included mice (20-30 g), rats (Sprague 

Dawley, Zucker Diabetic Fatty, Wistar-Hannover,) ranging from

<200 to 500 g, and adult beagle dogs. In addition to providing

blood samples at programmed timepoints, rodent studies also

included collection of urine and feces, and data on the rota-

tional behavior of the animal throughout the course of study.

Animals were either dosed with innocuous compounds not asso-

ciated with cardiovascular anomalies (acetaminophen) or those

known to cause QT interval prolongation in humans

(terfenadine). Blood samples were analyzed to determine phar-

macokinetic profiles of the drugs used and electrocardiograms

were taken prior to, during and after dosing to monitor changes

in the QT interval. The studies in dogs were continued for peri-

ods up to 4 h, during which 250 µL blood samples were taken

every 5 min. Studies in mice were conducted using collection of

up to nine blood samples (5 to 10 µL each) over 24 h. Rat blood

samples of 10 to 250 µL were taken at 12 points during a 24-h

period to establish pharmaco kinetics. Electrocardiograms were

taken automatically at intervals ranging from every 10 min. to

every 4 h and continuing for periods up to two weeks. The feasi-

bility of using automated blood sampling with concurrent

electrocardiograms to monitor pharmaco kinetics and cardio-

vascular changes has now been established for rats and dogs.

Automated blood sampling for mice has also been demonstrated

but electrocardiogram recording in this species was not at-

P25 Collection of Expired 14CO2 from the Nonhuman Primate 

Using Lexan Chambers in Conjunction with Stainless Steel/ 

Plexiglas Metabolism Cages

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The nonhuman primate is a widely used species in studies de-

signed to characterize the metabolism and excretion of a test

material. While such information is of central importance as it is

often used to aid in the interpretation of results from efficacy and

safety studies, the quality of such data is largely determined by the

quality of the equipment and procedures employed in the labora-

tory. Previously established techniques for measuring CO2 and

organic volatiles have possessed some undesirable characteristics

that have led to technical difficulties such as sample loss or undue

stress to the animal. The present procedure relies on the use of a

stainless steel and Plexiglas metabolism cage (0.5 m3) enclosed

within a sealed Lexan chamber (1.04 m3) in conjunction with a

unique circulatory inflow/outflow air pump system specifically

designed for this species. This unique caging system confers a

number of distinct advantages over previous methodologies. By

allowing the test animal to remain in its home cage, undue stress
to the animal as well as the likelihood of sample loss and sampling

error is reduced, enabling accurate and repeated quantitative

measurement of expired 14CO2 and organic volatiles.

P26 Development and Organizational Implementation of a Ca-

nine Functional Observational Battery (FOB) for Use in 

Regulatory Toxicology Studies

K Grant*, M Eliel, TJ Baird, MR Lane

Department of Pharmacology, MPI Research, Mattawan, MI

To satisfy recently adopted regulations outlined by the Inter-

national Conference on Harmonization of Guidelines for Safety 

Pharmacology Studies for Human Pharmaceuticals (ICH S7A), 

and existing general toxicology test guidelines promulgated by 

the FDA and other worldwide regulatory agencies, we have de-

veloped a systematic procedure for conducting neurobehavioral 

evaluations in canines. The beagle dog is a frequently employed 

non-rodent species in toxicology studies for which there is a large, 

established historical database. The canine is also a universally 

used test system for the evaluation of functionally toxic com-

pounds in safety pharmacology studies. For these and other 

practical reasons, a comprehensive series of neurobehavioral 

evaluations was adapted from previously published literature for
systematic application in fundamental nervous system assessment in the beagle dog. This poster outlines the procedures that were adopted in the development and validation of this canine FOB. This methodology offers an alternative test system for the neurobehavioral safety evaluation of new pharmaceutical and chemical entities. The index is suitable for independent safety (pharmacology) assessment, or inclusion as an integral part of general safety (toxicology) evaluation of investigational new drugs and new chemical entities.

**P27 Development and Validation of a Pulmonary Data Collection System for Use with Nonhuman Primates in the Conduct of Regulatory Safety Pharmacology Studies**

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Evaluation of the effects of a proposed pharmaceutical product for human consumption on the respiratory system is one of the core components of safety pharmacology studies, as indicated by the recently adopted ICH S7A guidelines. While validated rodent and canine models have come into common use in evaluating pulmonary response following the formal adoption of ICH guidelines by the Food and Drug Administration (FDA) and other worldwide regulatory agencies, the nonhuman primate has not represented a standard test system in regulatory safety studies. Given the universal employment of the nonhuman primate in general toxicology evaluations, there is a perceived need to characterize pulmonary positive control data in this species to establish the viability of this test system with respect to future pulmonary safety evaluations. ICH S7A guidelines for the conduct of safety pharmacology studies indicate the inclusion of two primary parameters, volume and respiratory rate, as fundamental to establishing the safety profile of a test article. We now report on the development and validation of a plethysmography method designed for use with nonhuman primates to collect such fundamental pulmonary function parameters. A computerized data collection system is designed to monitor and record pulmonary parameters from chair-restrained, nonhuman primates. This system is comprised of a plethysmograph and pressure transducer interfaced to a PC, via Gould signal amplifiers and hardware. Results indicate the sensitivity and reliability of this method in capturing selected respiratory endpoints for safety studies designed to fulfill regulatory guidelines for pulmonary safety evaluation.

**P28 Evaluation of Postsurgical Recovery for Rats in an Automated Blood Sampler Versus Rats Returned to a Home Cage**

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Intravenous catheters, and other devices such as microdialysis probes, are routinely implanted into rodents in our laboratory. After completion of surgery, animals are placed either in an automated blood sampler or returned to their home cage. In each case, the animal is kept warm until it regains consciousness and is then observed for several hours after surgery. Animals placed in the blood sampler often appear to be in better overall condition than those returned to a home cage when observed at intervals ranging from one to 24 h postsurgery. The purpose of this study was to determine whether a quantitative difference in the rate of recovery could be determined by measuring animal activity, fecal output and urine production. Subjective evaluations of general appearance, grooming, appetite and posture were also performed. Twenty Sprague Dawley male rats were introduced to the same type of caging system that permitted collection of animal activity data, urine and feces. Baseline levels of all three indicators were made prior to surgery. The cages were marked to identify the animal that had been housed there prior to that animal was removed for surgery. Jugular vein and femoral vein catheters were implanted under anesthesia, and a subcutaneous bolus of sterile saline was administered at the end of surgery to reintroduce fluids. The animals were then returned to their original cages. Half the animals were connected to the automated blood sampler, which then delivered a bolus of 20 µL of sterile saline every 12 min. Food and water was provided ad libitum. Animal activity recorded the rotational movement of the animals in the cage throughout the length of the study. Urine was separated from feces and collected in a refrigerated vial 6 and 18-24 h after introduction of the animal. Feces were collected after 18-24 h, dried under vacuum and then weighed. Subjective evaluations of animal status were made at 1 h, 4 h and 14 h after placing the animal in the cage. Both quantitative and subjective evaluations indicated that animals receiving the periodic infusion of saline regained their presurgical status sooner than those returned to the same cage without this infusion.

**P29 F344/N Rats as a Model to Study Bone Regeneration**

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The aim of this research is to construct an in vivo model in order to study different factors that have influence on bone regeneration, and to evaluate the efficacy of different membranes and disodium pamidronate on bone regeneration. Forty Fisher 344 (F344/N) male rats (300 g) were operated under general anesthesia with Ketamine/Xylazine i.m. Three defects 3-mm in diameter and 3-mm in depth were made on the femur. One collagen membrane and another expanded polytetrafluoroethylene membrane were placed over two of these defects; the third one remained untreated. Then, the animals were divided into two groups of 20 specimens each. Group 1 didn’t receive an additional treatment and Group 2 received 3.5 mg/kg/day of disodium pamidronate s.c. during 7 days. This way, we had a control defect (without membrane or drug) and five different types of treatment. After 30 days, the animals were sacrificed in order to process the tissue for histological examination. Histologic studies were performed with an imaging digital analyzer. All treatments showed higher bone regeneration than the control ($P < 0.001$ ANOVA On Ranks and Dunnnett’s Multiple Comparison Test). Additionally, the defects in Group 2 had better regeneration than the same defects in Group 1 ($P < 0.001$ Rank Sum Test). This in vivo model has shown to be viable and easy, and it permits evaluation of different aspects of bone regeneration. Additionally, the combination of membranes with disodium pamidronate have shown an important increase of regeneration rate, which opens a new way to treat structural bone defects.
Objective: To evaluate the growth and antigenic expression of a human bronchial adenocarcinoma xenografted in athymic nude mice. Methods: Xenografts: fragments of the tissue were implanted subcutaneously in the subaxial area of six 8-week-old athymic nude mice. Transplanted mice were daily monitored for tumor development and xenografted tumors were measured twice a week. Immunohistochemical analysis was performed following standard procedures; staining was evaluated according to positive reaction, intensity and distribution. Five human bronchial adenocarcinoma proved to be positive with the panel of monoclonal antibodies (MAbs) were included as positive controls while negative controls were incubated with phosphate buffer instead of MAbs. The expression of different antigens related to tumor invasion and metastasis were studied using the following MAbs: two anti MUC1 protein core: C595 (IgG3) and SM3 (IgG1); three anti-carbohydrate antigens: sialyl-Lewis x, KM93 (IgM), Lewis x, KM 380 (IgM), Lewis y, C14 (IgM), an anti-carcinoembryonic antigen (CEA) C365 (IgG) and an anti pan-cytokeratin antibody (SIGMA, USA). As second antibody, tissue specimens were incubated with anti-mouse IgG/IgM antibodies labeled with peroxidase. Results: Tumors developed at the inoculation site without invading surrounding areas with a latency period of about 30 days and tumor take rate yielded 85%. Histopathological observation showed a solid mass of large cells with clear cytoplasm. Immunohistochemical analysis found that tumors expressed all the antigens studied; MUC1 and Lewis y showed a diffuse and strong reaction; sialyl Lewis x and Lewis x presented a moderate staining while CEA showed a mild reaction. Cytokeratins showed a weak positive cytoplasmic expression. Conclusions: The method of engraftment employed proved to be useful since tumors grew successfully in athymic nude mice. Xenografted tumors were of epithelial origin, and they expressed tumor markers related to neoplastic invasion and metastasis.

P31 Human Breast Carcinoma Xenografted Tumor and Their Metastatic Implants in Athymic Nude Mice: A Comparative Study

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Objective: To characterize histopathological and immunological aspects of a human breast adenocarcinoma xenografted in athymic nude mice and their spontaneous metastatic implants. Materials: Patient tumor: an invasive ductal breast carcinoma with lung metastasis (disease stage IV); six female nude mice (6-8 weeks old); and Monoclonal Antibodies (MAbs) included: three anti MUC1 protein core: HMF51, C595, SM3 and four anticarbohydrate associated antigens: Tn hapten, Lewis y, Lewis x and sialyl Lewis x. Methods: xenografts: 2 x 10^6 cells were injected subcutaneously in subaxial area; transplanted mice were monitored for tumor development daily. Immunohistochemical analysis was performed following standard procedures; staining was evaluated according to positive reaction, intensity and distribution. Ten invasive ductal breast carcinoma positive with the panel of MAbs were employed as controls; negative controls were incubated with phosphate buffer instead of MAbs. Results: at 30-40 days of implantation tumors growth was observed at implantation site in five out of six mice. The necropsy of mice was performed; liver, spleen and lung showed metastatic implants. The examination of primary tumor and its metastasis revealed similar characteristics: invading malignant cells dispersed in masses while in some areas cells formed cords. By immunohistochemistry, the original tumor showed a positive antigenic reaction for MUC1, sialyl Lewis x, Lewis x and Lewis y antibodies while Tn hapten was negative. The tumor, which develops subcutaneously in mice and its liver implants, expressed MUC1, sialyl Lewis x, Lewis y and Tn hapten but failed to express Lewis x. Spleen metastatic implants were reactive with anti MUC1, Tn hapten and sialyl Lewis x; finally lung implants showed MUC1 and Tn hapten expression. Conclusions: A novel metastatic nude mice model for breast cancer was achieved; also, a different antigenic expression was found among human primary tumor, mice tumor and its metastasis.

P32 Lack of Gravitational Force (+Gz) Effect on the Over-the-Wire Stainless Steel Greenfield IVC Filter in Swine

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Objective: Although more than 100,000 Greenfield inferior vena cava (IVC) filters have been placed in the past 25 years, the maximum safe +Gz exposure for patients with Greenfield filters is unknown and there are no recommendations with regard to exposure to +Gz for patients with this device. The purpose of this study was to determine the effect of exposure to high +Gz on in vivo Greenfield IVC filters.

Methods: Fifteen pigs underwent venous cut down and placement of a stainless steel Greenfield filter. A 4-week observation period simulated realistic convalescence and allowed sufficient time for epithelialization. Ten pigs were exposed to acceleration stress in a centrifuge (3G run for 15 sec. followed by rest until return to baseline heart rate, then a 9G run for 15 sec.), with inertial loading in a head-to-tail direction (+Gz). Fluoroscopy during acceleration stress allowed assessment for filter migration. Five pigs were not exposed to acceleration stress. AP and lateral abdominal radiographs were obtained post-filter placement, convalescence, and centrifuge exposure to determine the position and integrity of the filter. All 15 pigs were then necropsied and the IVCs resected and evaluated for gross or histologic pathology.

Results: IVC filter placement was technically successful in all 15 pigs. Radiographic measurements were limited due to differences in pig positioning. Fluoroscopy showed no filter migration. There was no gross evidence of perforation or hemorrhage. The attachment hooks and approximately 2 mm of the tines were securely embedded in the wall of the vena cava. The tips of the hooks minimally penetrated the wall, extending into the periadventitial fibro-adipose tissue and anchoring it externally.
to the vein. There were varying degrees of fibroplasia involving the hooks and tip of the filters in both the control and experimental groups. All 15 filter attachment sites were similar histologically, exhibiting stretching of the tunica media and adventitia, subacute inflammation and fibrosis associated with the implant, dilation of small capillary vessels near the implant, slight amounts of hemosiderin near the implant site, and free erythrocytes within the lymph node sinuoids.

Conclusion: Greenfield filter position and vena caval morphology at the implantation site is unaffected by high acceleration stress.

P33 Postnatal Exposure to Nicotine and Alcohol Using a Non-invasive, Minimally-Stressful Model in Rats

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Nicotine is considered the gateway drug for other drugs of abuse, since nicotine exposure through cigarette smoking is one of the first drugs routinely self-administered by humans. In addition, in women of child-bearing age, alcohol routinely is consumed concurrently with smoking. Nicotine and alcohol (EtOH) each exert profound effects on brain development, yet the consequences of exposure to both have not been well studied, despite strong epidemiological evidence for co-morbid effects. Secondly, the critical human brain growth spurt occurs entirely in utero during the third trimester of pregnancy; however, this occurs postnatally (days 0-14) in rats. Therefore, to investigate the outcome(s) of co-morbid exposure that pertains to human brain development using a rat model, nicotine and EtOH delivery should continue postnatally. Current models of postnatal nicotine and alcohol exposure require intraperitoneal injections for nicotine and an artificial rearing environment with an intragastric feeding tube (‘pup in a cup’ model) for EtOH, both of which are significant stressors. However, stress and/or maternal separation have profound effects on behavioral outcomes. Therefore, we developed a non-invasive, minimally stressful paradigm that used the natural suckling reflex to manually administer EtOH (4 g/kg), and nicotine was delivered via nursing from the dam with a nicotine-containing miniosmopump (8 mg/kg/day). Pup blood nicotine levels (BACs) peaked (144 ± 11 mg/dL; n = 45) at +60-90 min., consistent with the pregnant dams’ peak BACs of 163 ± 25 mg/dL (n = 8) at +90 min. and comparable to peak BACs characteristic of the pregnant dams’ peak BACs of 163 ± 25 mg/dL (n = 8) at +90 min. and comparable to peak BACs characteristic of the pregnant dams’ peak BACs of 163 ± 25 mg/dL (n = 8) at +90 min.

P35 The Peromyscus Genetic Stock Center: New Approaches to Old Problems with Development of a Molecular Map

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The deer mouse (Peromyscus maniculatus) and congeneric species are the most common native North American mammal, ranging from Alaska to Central America. Living in a diversity of habitats, these mice constitute a major component of Neartic terrestrial ecosystems. Laboratory stocks of both wild type and genetically variant Peromyscus are used for investigations in which laboratory-based studies can be interfaced with those of natural populations. The Peromyscus Genetic Stock Center, established in 1985, provides a reliable source of these animals and related materials to the national scientific and educational communities. The Center currently keeps seven species of Peromyscus and more than 35 distinctive mutant and other genetically defined stocks, primarily of the deer mouse. The Stock Center also supplies biological materials including fresh, frozen and preserved tissues and molecular probes and libraries. The center functions as a clearing house for information regarding this genus by sponsoring an internet database (PeroBase), home page, and the semi-annual Peromyscus Newsletter. The Stock Center will soon be the focus of a major effort involving a number of investigators to establish an intermediate density genetic map, relying heavily on PCR-based genetic markers such as microsatellites, RAPDs, and ESTs. A set of genome-wide radiation hybrids is being developed to aid in linkage analyses. The considerable effort devoted to this project will benefit members of the Peromyscus research community interested in identifying and studying genes involved in a variety of processes including speciation, genome imprinting, disease susceptibility, and behavioral and physiological adaptation to habitat.

P36 The Real and Virtual Use of Laboratory Animals to Teach Forensic Sciences

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Currently, there are no virtual forensic entomology and anthropology mock crime scenes. We currently are documenting mock crime scenes to be able to create these virtual crime scenes to provide this training as well as attempting to minimize the need for animals. This presentation will present both the practical aspects of the mock crime scenes as well as our efforts to turn these experiments into virtual crime scenes. The mock crime scenes
Alzheimer’s Disease (AD) occurs when neurons in the memory and cognition regions of the brain are accompanied by the accumulation of the long amyloid β-proteins of the 39 to 43 amino acids derived from the amyloid precursor protein (APP) by cleavage with β- and γ-secretase. In general, an increased production of Aβ-42, by mutation of PS2 genes, promoted caspases expression, and is associated with the Cox-2 found in the brain of AD patients. To address this question in vivo, we expressed the human mutant PS2 (hPS2m) (N141I) as well as wild PS2 (hPS2w) as a control in transgenic (Tg) mice under the control of the neuron-specific enolase (NSE) promoter. Water maze tests were made to demonstrate the behavioral defect, and Dot blot, Western blot and immunohistochemical analyses were also performed on the brain, with the hPS2, Aβ-42, caspases-3 and Cox-2 antibody. We concluded that i) Tg mice showed a behavioral dysfunction in the water maze test; ii) levels of hPS2, Aβ-42, caspase-3 and Cox-2 expression were modulated in the brains of both Tg mice; iii) dense staining with antibodies to hPS2, Aβ-42, caspase-3 and Cox-2 were also visible in the brains of Tg mice, compared to age-matched control mice; and iv) there were not appeared the distinguishable ADs phenotypes between hPS2w- and hPS2m-Tg mice. These results suggest an elevation of Aβ-42, by over-expression of hPS2 and mutation of hPS2m, might induce the behavioral deficit as well as the caspases-3 and Cox-2 induction, and these could be useful in the therapeutic testing of compounds to have considerable clinical effects.

P38 Continuous Glucose Monitoring in Spontaneous Diabetic Monkeys

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Spontaneous diabetic cynomolgus monkeys (Macaca fascicularis) exhibit a condition similar to human type 2 diabetes and are useful for evaluating the efficacy of new anti-diabetic compounds. These monkeys were maintained by treatment with insulin at a dosage level fixed based on fasting blood glucose levels and HbA1c values, which are determined periodically by blood sampling. Recently, the continuous glucose monitoring system (CGMS: MiniMed, Sylmar, CA), which takes glucose measurements continually for 24 h, was purchased. Therefore, we determined whether the use of this system could help in monitoring the glycemic condition and provide a better regimen of insulin treatment in diabetic monkeys. The CGMS was attached to and fastened on a total of five diabetic monkeys and their blood glucose profile was obtained. The CGMS took accurate measurements, with a median correlation of 0.95 and a mean absolute difference of 8.2 ± 4.7% in comparison with the handheld blood glucose meter. The diabetic monkeys were monitored two or three times during a 3-month study period. Throughout the study, the feeding time, dosage and insulin administration time were changed in three monkeys out of five based on the results of each monitoring. HbA1c levels were measured before and at 1 and 3 months after insulin administration. Although the change in daily insulin dosage was not significantly different, HbA1c levels were decreased from 7.6 ± 1.3 to 6.5 ± 1.1% (P < 0.05) at the end of the study. It was concluded that the values of CGMS well matched those obtained with the handheld blood glucose meter. Using CGMS in studies to elucidate blood glucose profiles allows the blood glucose levels of the monkeys to be monitored during the night as well as the day. Therefore, such continuous monitoring is useful in preventing nocturnal hypoglycemia and could lead to the successful management of diabetes.


E Tena*, D Herrera, E Foyo, P Bravo, CA Tena, S Beltrán, J Calderón


Developmental dysplasia of the hip (DDH) is the common term used to describe a condition in which the femoral head has an abnormal relationship to the acetabulum. In human infants this syndrome includes frank dislocation (luxation), partial dislocation (subluxation) and instability of the hip, as well as an array of radiographic abnormalities that reflect inadequate formation of the acetabulum, condition reported as frequently occurring in different animal models due to complex hereditary factors. In the present study, a small group of laboratory rabbits (Oryctolagus cuniculus) with a known carrier genetic history of Splay Leg were selectively mated, to produce an offspring of 5:2 dysplastic animals. The affected rabbits exhibited gross evidence of physical abnormalities, including splaying of one or more legs, impaired locomotion and limited abduction indicative of acetabular involvement as early as 9 weeks old, and therefore were radiographically studied at 70, 120 and 180 days in order to assess pelvic morphological deviations, that were comparatively studied to define the analogies and extent of lesions shown by human infants less that a year old, using also clinical maneuvers for assessing hip stability as the Ortolani and Barlow tests and indicators such as the Acetabular Index (AI), Wiberg Angle (WA) and the Femoral Head Extrusion Index (FHEI). The comparative study of the selected radiographic cases demonstrated progressive bilateral dysplastic acetabular changes, with an AI of 34°, a negative WA of –2° and a FHEI of 2 mm, lesions leading to a complete in vivo dislocation considered as a late sequela. All morphologic changes noted were consistent with DDH of the chosen infant cases, including a variety of physical disorders such as asymmetric thighs or buttock creases, true shortness of legs and limited abduction present on both analyses. Finally, this clinical study has illustrated that the induced condition in the rabbit shared similar patterns of familial tendency inheritance toward hip dysplasia, with more females affected than males, analogous acetabular maldevelopment with a severe anteversion of femoral neck and...
subsequent hip instability, as well as significant evolutive differences among humans infants and rabbits.

P40 Refractometry for Quality Control of Anesthetic Drug Mixtures

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For reasons of safety, injectable anesthetic drugs used in rodents must often be diluted to increase the accuracy and convenience of dosing. Conventional analytical methods of assessing dilution and compounding accuracy are expensive and time-consuming. Clinical refractometers have been used by hospital pharmacies to detect illicit diversion of some narcotic drugs, and this method was examined as an in-house means to establish quality control of certain anesthetic drug mixtures. Refractometric properties of each drug tested were evaluated using primary dilutions of the drug with distilled water at 100%, 75%, 50%, and 25% of labeled concentration, with a zero point established with distilled water. The refractive difference from water at each concentration was graphed and the resulting curve examined. For ketamine, xylazine, acepromazine, buprenorphine, and medetomidine the results confirmed a simple linear relationship between concentration and refractive readings. Because the refractive readings for single drug concentrations are additive, the standard curve for each drug can be used to predict the refractive index of various drug combinations. Known drug mixtures were prepared in correct ratios and in various incorrect ratios to evaluate the ability of this method to detect common mixing errors. The results show that mixing errors can be detected refractometrically. The method requires that the user know the identity of the drug(s) and diluents in the mixture and that the refractive readings fall within the upper and lower limits of the instrument.

P41 Application of Reduction through Real-Time Imaging

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Mice are one of the most frequently used animal models for research investigations of human disease and are particularly well suited for the development of models of human cancer. Typically, human tumor cells are injected into immunocompromised mice in order to establish either a localized tumor or metastasis to distal tissues or organs. In the case of localized tumors, physical tumor measurements can be made on a periodic basis to determine growth or regression; however, assessment of molecular or biological activity of potential antitumor products generally requires sacrifice of the animal and collection of tumor and relevant tissues. In the case of metastatic or orthotopic tumors, quantitating the tumor burden is more difficult and usually involves sacrificing the animal. In addition to the analytical limitations of mouse tumor models, the incidence of tumor take and spontaneous regression must be factored into the design of each study, often requiring a significant increase in the number of animals placed on study to yield statistically valid results. In contrast, when luciferase-transfected tumor cells are used, analyses of local and metastatic tumor progression can be performed on live animals at multiple timepoints during the course of the study using a whole animal imagining system such as the IVIS™ Imaging System developed by Xenogen. The technology of this system is called “in vivo biophotonic imaging” and measures the light emissions that occur when luciferin is used as a substrate by luciferase. Luciferin is administered to the animals shortly before they are to be imaged, and images can be collected for up to 30 min. following preparation. This analytical tool provides the research scientist with the ability to track biological activity in real time at the molecular level in a living mammal. Furthermore, this method can dramatically reduce the numbers of animals necessary to provide statistical significance. At Genetic Therapy, Inc. we have demonstrated a significant reduction in the number of animals used on certain types of tumor burden studies by using this imaging system.

P42 A Comparison of Brain/Plasma Ratio With and Without Perfusion in the Male SD Rat Using Diazepam as a Marker

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Lipophilic compounds are of interest when targeting specific receptors in the brain. Not all lipophilic compounds are able to cross the blood: brain barrier. Brain/plasma (B/P) ratios obtained from a rodent pharmacokinetic assay are useful in helping determine which compounds are brain-penetrable. The present study was performed to determine if whole body saline perfusion for complete blood removal was required to accurately measure brain tissue compound levels. Diazepam was used as a positive control since it is highly brain-penetrable. Following intravenous dosing with diazepam, rats (300-400 g, male Sprague Dawley, n = 20) were anesthetized, blood was collected, then the brain was removed following no perfusion or whole body perfusion with saline. The analyte was recovered from plasma or brain homogenate by liquid-liquid extraction and subsequently analyzed by liquid chromatography/tandem mass spectrometry (LC/MS/MS). The B/P level ratios determined using LCMS were not significantly different in perfused versus non-perfused rats (P ≤ 0.05). Whole brain collected from non-perfused, male SD rats is therefore an acceptable practice for the determination of B/P ratios. This approach has markedly reduced the technical time required to generate B/P ratio data due to elimination of the requirement for anesthesia and surgical preparation of animals.

P43 A Comparison of Two Methods of Bile Duct Cannulation in Rhesus Monkeys (Macaca mulatta)

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Pharmacokinetic characterization of novel compounds often requires the determination of bioavailability, intestinal absorption and hepatic clearance. These studies often require access to the biliary system. Two techniques were evaluated for the collection of bile from conscious, adult rhesus macaques. In both methods, bile was collected from the common bile duct. A commercially available T-tube system was surgically implanted into the common bile duct of eight animals. In five other animals, a 5 french heparin-coated cannula was surgically implanted into the common bile duct through the cystic duct, and a 5 mm vascular occluder was placed around the distal aspect of the common bile duct. Both systems used a subcutaneous access port for bile sample collection. Complete blood counts, serum chemistry analyses, bile flow rates, and imaging techniques (fluoroscopy and radiography) were used to evaluate each animal prior to use in any pharmacology studies. Periodic liver function tests (BSP clearance) were also conducted. The bile duct cannulas remained functional for 150-360 days. Although the compari-
son between the two systems is ongoing, both methods of bile duct cannulation are effective for bile sampling and useful in determining both metabolism of parent compound and the rate of hepatic clearance.

**P44 A Cost-Effective Method of Daily One-Hour Infusion in Rats**

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Intravascular infusion studies often present many challenges to the researcher and can be performed using a variety of methods. Tail vein infusion is a simple, yet labor-intensive, technique often used in short-term studies in rats. However, for instances where the compound is known to cause tissue irritation, access to the central venous system allowing more rapid dilution of compound is a more humane alternative. To perform a 1-week intravenous infusion study using 32 rats receiving daily, 1-h infusions, we developed this alternative to continuous infusion. This method avoided the added expense of costly swivel apparatuses and the purchase of additional pumps. A vascular access port (VAP) was surgically implanted to allow access to the jugular vein. To make the infusion assembly, a non-coring (Huber) needle was inserted into the tubing of a modified butterfly catheter and the luer opening was closed with an injection cap. The infusion assembly fit snugly into a Covance Infusion Harness' and the Huber needle, which was secured to the animal’s body by the harness, was inserted into the VAP. The harness apparatus remained in place for the duration of the study. This allowed easy access to the VAP with minimal daily preparation and decreased stress to the study animals. Furthermore, the harness ensured that the Huber needle stayed in place, thereby minimizing the chance of dosing error. After the 1-h infusion, the VAP was locked with heparinized saline. To restrain the animals, a dosing chamber was fabricated to facilitate the infusion of 12 rats simultaneously. The design of the multi-compartmental chamber provided a virtually stress-free environment for the animals that was small enough to fit on a bench-top setting. This poster will illustrate a novel, low-cost alternative for performing intravenous infusion studies in rats, which is both technician-friendly and humane for the animals.

**P45 A Jacketless Tether System: A Novel Approach to Canine Comfort during Chronic Infusion Studies**

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Covance Laboratories Inc. is continually looking for ways to improve our animals’ comfort and well-being while under our care. Both ambulatory and tethered systems are currently practiced for long-term chronic and long-term intermittent infusion as well as sample collection studies. Both systems require that the dogs wear some type of collar to prevent access to the protected infusion lines. Recently we have developed a method for chronic infusions that eliminates the use of jackets by simply attaching the tether directly to the collar (LOMIR Biomedical Inc.). The tether acts much like a leash and provides increased mobility while reducing the possible stress related to wearing a jacket for extended periods of time. In addition, sterile dressing, wrapping, and taping of the catheter exit site are more easily accomplished in the neck region than on other more typical catheter exit sites or vascular access port locations (i.e., the scapular or lateral thoracic region). Eight beagle dogs were surgically prepared with jugular catheters that were externalized at the lateral cervical area and then placed on the jacketless tether system. The study durations ranged from 4 to 6 weeks. All animals adapted well to their collars. There were no remarkable clinical or anatomical pathology results. Clinical observations were limited to some minor abrasions of the skin under the collar. This new system was preferred by all of the infusion experienced technicians that used it. The method also lends itself for use with other possible procedures or species (e.g., arterial access, LVP pressure, hardwiring direct ECG or EEG leads, rabbit reproductive infusion studies). A second generation of collar has been developed to eliminate skin abrasion, provide greater comfort and additional room for a vascular access port and infusion line loops or connections. The jacketless system being used at Covance Laboratories—Madison provides a comfortable alternative for chronic and long-term intermittent infusions in the dog.

**P46 A Novel Restraint Device for Use in Administration of Infectious Agents in Rodents**

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Administration of an infectious agent into rodents poses challenges for the safety of laboratory personnel. A novel restraint device has been designed for maximum control over the animal while shielding the fingers, wrist and lower arm of the technician. The device and associated techniques were reviewed and approved by the Institutional Biosafety Committee. The Plexiglas device offers many safety advantages over manual restraint or conventional rodent restrainers while still allowing for easy disinfection. The shield sits on a 7.0 × 10-in. base. The Plexiglas shield stands 5.5 in. high and 9.5 in. wide, with a 4.0-in. Plexiglas sash that tightens into the desired position (and can accommodate animals of varying sizes). The shield has a 3.0-in. lip on top to protect the injector’s arm or hand, but allows for free movement. In a study using transgenic mice, 30 animals were sedated with carbon dioxide. The retro-orbital sinus was chosen as the route of administration of an infectious agent. The device offered stabilization and positioning of each mouse for injection, and prevented any accidental needle sticks. The device can be used for various routes of drug administration.

**P47 A Novel Restraint Unit for Cynomolgus Monkeys**

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In toxicology laboratories, it is often necessary to restrain and dose nonhuman primates daily in order to assess the effects of novel therapeutic compounds. Dosing and other manipulations such as serial phlebotomy for blood drug levels and indirect blood pressure measurements should be carried out on nonsedated animals whenever possible. The method used for these manipulations must be humane, rapid, and safe for both the animal and caregivers, providing for a minimum of physical contact between the nonhuman primate and human handler. Pole and collar techniques can be effective; however, additional chair restraint may be required for oral dosing, animals and handlers can sustain inadvertent injury during their use, animals may develop dermatopathies at the collar site, and the system may be unwieldy for some animal care attendants to use with larger animals. This laboratory has refined a mobile restraint squeeze unit that works well with cynomolgus monkeys and does not require
collars. The unit consists of adjustable tubular steel bars set on a base with locking castors, and is durable, readily sanitized, safe, and easy to use. The unit requires only a transfer cage that has been modified to hook onto the restraint unit, permitting the monkey access through a guillotine door that can be locked closed. The device can also be used to physically examine nonsedated animals closely and provide clinical treatments as necessary. Animals acclimate readily to this restraint method and it provides an alternative to the pole and collar technique. Use of this unit has increased the ease, efficiency, and safety of working with cynomolgus macaques in our laboratory while providing humane restraint for the animals.

P48 Comparison of Single Drug Administration to Multiple Drug Co-Administration in Drug Discovery

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We evaluated brain penetration with 30 different compounds from the same discovery program. In an attempt to increase throughput in our screening efforts and reduce the number of animals required for a study, mixture dosing was evaluated. Results from single compound administration were compared with results following administration of a mixture of four compounds. The analyte was recovered from plasma or brain homogenate by liquid-liquid extraction and subsequently analyzed by liquid chromatography/tandem mass spectrometry (LC/MS/MS). Preliminary results, with specific classes of compounds, show no major differences (ranking order) in brain or plasma concentrations between mixture dosing and single compound administration, suggesting that mixture dosing could be applicable to brain penetration studies in the drug discovery phase. This approach reduced the total number of animals required for this type of study by 75%.

P49 Cost-Effective Topical Anesthetic for Auricular Blood Collection in Rabbits

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Blood collection in rabbits is a common task associated with polyclonal antibody production. The auricular artery can be used to collect volumes of blood up to 10-20 ml. In our facility, acepromazine maleate (1.0 mg/kg) is used for blood collection procedures because of its vaso-dilation properties and for mild tranquilization of the animal. In addition, we also prefer to use a topical anesthetic to desensitize the ear and allow needle penetration without a reaction. EMLA cream (lidocaine 2.5% and prilocaine 2.5%), a human product purported to penetrate in intact skin other than mucous membranes, has been used in our facility for topical anesthesia the ear. Although EMLA cream is effective, it is very expensive, requires a thick application of cream at the penetration site and needs an extended contact time (30 min.) for successful desensitization. Another topical anesthetic cream that is often used to desensitize mucous membranes is 2% lidocaine hydrochloride jelly (Xylocaine). We compared the effectiveness of topical application of 2% lidocaine jelly to that of EMLA cream for auricular blood collection. Rabbits were bled as part of their routine antibody production protocol using acepromazine in combination with EMLA or lidocaine cream and compared to rabbits bled with EMLA or lidocaine cream alone. Rabbit responses to venipuncture were scored by a single individual using a simple scoring scale. Results indicate that topical administration of a small amount of 2% lidocaine hydrochloride jelly in combination with acepromazine can be an equally effective alternative to EMLA cream in desensitization of the ear for blood collection.

P50 Evaluation of Enterohepatic Recirculation of CT20026 following Oral Administration in Bile-Linked Rats

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We evaluated the extent of enterohepatic recirculation (EHR) of CT20026 using a bile-linked rat model. In a pilot study of the model, a donor and an acceptor (control) rat were linked via bile ports as follows: the bile flow from the donor rat was diverted to the duodenal port of the acceptor rat and the bile flow from the acceptor rat was diverted to the duodenum of the donor rat. Thus, the bile links between the two rats served as a continuous closed circuit and would allow for the secretion/reabsorption cycle of the test compound to occur. We found that by placing the donor rat in a cage approximately one foot higher than the acceptor rat, gravity would assist with the flow of bile from the donor rat to the acceptor rat. In addition, the pilot study was performed in conscious animals because we found that anesthesia slowed the bile flow to an extent that no appreciable bile exchange occurred. In the experimental study, bile duct ports between two rats were linked similarly as in the pilot study. Following oral administration of CT20026 to the donor rats, blood samples from both donor and acceptor rats were collected at intervals over an 8-h period to determine the concentration of parent compound and/or its metabolites. Analysis of the samples showed that the blood of the control rats contained no significant amounts of CT20026 or its metabolites, therefore indicating EHR of the drug had not occurred. These findings demonstrate that the bile-linked rat model is a useful tool for evaluating the possibility of EHR, and how this could impact net systemic exposure and ultimately whether the drug should be considered as a long-term therapeutic.

P51 Evaluation of Sonomicrometer Measurements of End Diastolic Volume as an Index of Cardiac Preload in an Ovine Model

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The major factors affecting the heart as a pump are preload, afterload, contractility and heart rate. Preload is usually estimated clinically as the pulmonary capillary wedge pressure or as the central venous pressure. However, a variety of factors, such as changes in intrathoracic pressure, pulmonary disease, and/or valvular heart disease, may invalidate these measurements. Alternatively, preload can be measured as end diastolic volume (EDV). In order to evaluate the left ventricular EDV as an index of preload, 3 male sheep, 30-50 kg, were chronically instrumented with endocardial sonomicrometer crystals on the anterior-posterior (AP) and base-apex (BA) axes of the left ventricle and with an ultrasonic transit-time flow probe on the aortic root. Preload was varied by controlled hemorrhage followed by resus...
citation. Both 2-axis ellipsoid and 1-axis spheroid models were used to estimate left ventricular EDV and end-systolic volume (ESV). The ellipsoid estimate of cardiac output [Heart Rate × (EDV-ESV)] was linearly proportional to the ultrasonic flow probe cardiac output if the projection of the AP and BA axes intersected on the plane defined by the anterior, posterior and base crystals. The AP spheroid model was linear regardless of intersection. Sonometric measurements are a valid index of preload if expressed as percent of baseline EDV. Ejection fraction was preload-dependent and therefore was not a valid index of contractility with changes in blood volume.

P52 Intrathecal Catheter Placement for CSF Sampling

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A procedure to collect cerebral spinal fluid (CSF) from seven pigtail macaques (*Macaca nemestrina*) was developed as a component of a pharmacokinetic protocol. The following describes procedures used for intrathecal catheterization and CSF collection from the area of the foramen magnum through a lumbar approach. Animals were anesthetized with isoflurane inhalant anesthesia. Body temperature, heart rate, respiratory rate, saturated O₂, and end tidal CO₂ were monitored throughout the procedure. Anesthetized animals were positioned in sternal recumbency over a 20-cm diameter cylinder to spread the dorsal aspects of the lumbar vertebrae. Using aseptic techniques, the skin over the lumbar spine was surgically prepped and draped. The intrathecal catheterization technique involved palpating the spinal lumbar region at the lumbosacral junction. Using the iliac crests for orientation, the spinous processes of L-4 or L-5 were identified. A Toughy-Schiff (T-S) epidural needle with stylet in place was introduced perpendicularly just distal to the spinous process and carefully advanced. A “gritty” feeling and then a “give” was encountered when the needle was properly inserted. The stylet was removed to check for CSF; if no flow, the stylet was replaced and the needle advanced until a “pop” sensation was felt. An involuntary leg reflex was commonly elicited when needle was in the intrathecal place. After proper T-S needle placement, a flexible nylon catheter was inserted through the needle. The catheter was advanced as far as possible to the area of the foramen magnum. After catheter placement the T-S needle was removed. A 1-ml syringe was attached to the catheter and secured to the animal. The animal was removed from the cylinder and placed in lateral recumbancy. Seven 0.5-ml CSF samples were collected over 5 h. The catheters were removed at completion of sample collection. Postprocedure analgesia was administered. No complications or adverse effects were observed in the animals during or following the procedure.

P53 Minimal Restraint Chair and Muzzle for Nonhuman Primates

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Commercial restraint chairs for nonhuman primates are designed for short-term use and are rather generic in nature. Our cynomolgus monkeys (*Macaca fascicularis*) were constantly escaping from them and/or breaking them. We needed a chair specifically for our specialty use in glaucoma research. Minimal restraint is required, the animal must be comfortable for up to 10 h with its head on a level for easy access by study technicians, the chair must be adaptable to the pole-and-collar technique, and environmental enrichment must be practicable. Since we work around the eyes of conscious animals, we also needed protection from bites. A restraint chair was designed based on normal sitting posture and behavior of cynos. It consists of a metal pole frame on casters with a grid platform perch and a clear hinged top plate that contains a slotted groove for the collar. The animal is restrained in the chair only by the collar and a unique locking plate practically assures no escape. The animal’s head in the chair is about 4.5 ft off the floor, within comfortable reach of the technician. An access port, foot rests, and bars are incorporated for environmental enrichment. A mesh wire muzzle, in three sizes, with adjustable Velcro strap was devised to fit the various monkey faces. Over 100 animals have been trained to enter and remain calmly in these chairs for several hours and to wear a muzzle for up to 5 min. Both chair and muzzle have been used extensively by other groups for dosing and for routine medical procedures. No bites have occurred, and only one escape, since the chair and muzzle were designed in 1989. Our designs are an advance in safety for both animal and animal technician.

P54 Multiple Sensory Stimuli Enrichment for Chaired Monkeys

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Nonhuman primates are stressed when restrained in chairs because they are in a situation that is not “normal,” they have no control over where they are or what other animals they have to see and hear, and there is extremely limited opportunity for species-specific behaviors. Our research makes use of IACUC-approved protocols that allow chair restraint of groups of cynomolgus monkeys (*Macaca fascicularis*) for periods up to 10 h during studies and while the animals are being trained for these studies. Our monkeys are part of a long-term colony and we seek to give them the highest quality environment possible, both in their cages and during studies. The In Vivo Pharmacology Unit has devised an extensive series of chair-friendly enrichments that run the gamut of sensory stimuli: sight, sound, taste, smell, and touch. Some are simple foodstuffs with complexities of color, texture, and variety added. Others are elaborate devices that require time and curiosity and lots of manipulation. All encourage the animals to actively participate in some aspect of foraging, playing, grooming, and/or curiosity gratification. They include: baby toys, Kong toys, Nyla toys, two to three different forage feeders, frozen food treats, ball puzzles, and much more. We will list and show our current collection of enrichment techniques and devices, many of them in use with our specially designed primate chair. Anecdotes and data support that these enrichments during chairing make our monkeys cooperative and easily trained, reduce or supplant self-directed behaviors, extend the productive research lives of the animals, and reduce anxiety in novel situations. Future directions for enrichment of chaired animals will be explored.

P55 Percutaneous Access of the Anterior Tibial Artery for Direct Blood Pressure Measurement in the Macaque

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Pharmacia—Kalamazoo maintains a colony of cynomolgus macaques (*Macaca fascicularis*) implanted with telemetry equipment for safety pharmacology studies. Previous protocol required implantation of a vascular access port (VAP) into the femoral ar-
Chronic Ischemic Model

The methodology used to perform percutaneous access of the anterior tibial artery in conscious, restrained cynomolgus. Examples of data obtained from these animals are illustrated and summarized. Percutaneous access to the anterior tibial artery has replaced the use of VAPs in our facility for calibration of implanted telemetry devices.

P56 Restraining Device for Canines

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Canine models are frequently used in preclinical studies. Routine tasks such as imaging, dosing and sample collection can be difficult for the investigator and uncomfortable for the subject. Nuclear imaging studies, in particular, are often long and require the canine to be immobile. Chemical restraint may minimize stress and mobility, but alteration of normal biological and physiological effects preclude use of this method. The E.CAM is a Single Photon Emission Computed Tomography (SPECT) camera that is widely used for imaging. When the camera is used in a medical setting, verbal instructions can effectively be given to subjects. Naturally, verbal instructions do not work with the majority of canines used for research. It is therefore necessary to use a restraining device. Acclimatization to the device and training become important factors. A fabric body wrap supplied with the E.CAM is effective for human use. However, it was not sufficient to restrain the animals as they could maneuver out of it. An alternative restraining device was subsequently made from lightweight, durable Plexiglas. The innovative device, designed for comfort and flexibility, included Velcro straps, foam padding, nylon screws, and wing nuts. These materials were used because the E.CAM collimates gamma rays from radioisotopes. Many materials block or severely attenuate the passage of gamma rays. Use of Plexiglas allows for adequate collection of the rays. Additionally, the animals need to be comfortable while avoiding any undue stress. The device is fully adjustable to accommodate various size canines. Animals undergo training sessions that allow for a gradual acclimatization to the device. The same staff members conduct sessions so that the animals become familiar with them. Training session start with the animal being in the device 10-15 min. at a time, three times a week. As the animal progresses, the time is increased from 15 to 30 min., then 30 to 45 min. until 1 h of restraint is achieved. Each animal undergoes restraint training for a 1-month period and is then evaluated to see if they are a good camera candidate. The device and method used to train the animals has proven to be a success. Use of this device has increased the number of studies performed while allowing a higher quality of image to be acquired.

P57 Technical Considerations for Coronary Perfusion Validation Studies in the Hostile Environment of Magnetic Resonance Imaging Using a Chronic Ischemic Model

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Magnetic resonance imaging (MRI) is a modality with increasing clinical use in the assessment of cardiac functional and coronary perfusion. However, the large fringe magnetic fields of 1.5 Tesla MRI systems and sensitivity of ECG-gated, echo planar imaging techniques to radio frequency interference precludes use of standard laboratory procedures and equipment for validating MRI coronary flow and flow reserves. This study presents the technical consideration for performing coronary perfusion validation studies for baseline and hyperemic challenges in a swine model for chronic ischemia (n = 16, female Yorkshire swine, 25-36 kg). Titanium jacketed amered constrictors (2.25 mm, Research Instruments SW, Escondido, CA) were surgically implanted on the proximal left circumflex coronary artery. MRI assessments of coronary perfusion were compared with neutron activated microsphere methods (BioPAL, Worcester, MA) at baseline and during hyperemia prior to implant and at 1, 4 and 6 weeks postimplant. Selective coronary angiography was performed to confirm chronic constriction. Each MRI measurement of coronary perfusion was obtained under isoflurane anesthesia (2%) during a simulated breath hold (ISOTEC 4 Surgivet, Waukesha, WI). 7.0 × 10^6 microspheres diluted to 6 cc were bolus injected into the left ventricular cavity using a six hole 5F angled pig-tail catheter (Cordis, Miami, FL) which was introduced into the right femoral artery prior to transport for imaging. Catheter positioning within the left ventricle was validated prior to microsphere injection by hemodynamic monitoring. Reference blood samples were obtained at baseline and during adenosine (140 μg/kg/min) infusion. Microsphere injections were performed while the animal was in a steady state within the MRI magnet and reference blood withdrawn at 3.0 ml/min. for 3.5 min. Stable coronary perfusion during adenosine-induced hyperemia was demonstrated in separate experiments for periods ≥ 20 min. This study demonstrates a successful approach for performing coronary perfusion validation studies for MRI perfusion imaging.

P58 Collection of Multiple Samples from a Chronic Rhesus CSF Model

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A novel method of collecting cerebrospinal fluid (CSF) was developed for multiple sampling from rhesus monkeys that were instrumented with a chronic vascular access port (VAP). CSF is often analyzed to detect analyte and drug compound levels in neuroscience research. A catheter was placed 1 cm into the cisterna magna. The catheter was attached to a VAP that was secured subcutaneously. The CSF was collected by accessing the port by means of a Huber needle left open to allow the fluid to drip into a collection tube. This system enabled CSF samples to be collected at multiple timepoints while the animals were restrained in a primate chair. In order to maintain patency of this model, the animals were accessed three times a week. The skin was prepared aseptically prior to placement of the Huber needle. Approximately 1 ml of CSF was collected at each maintenance. The flow was timed to measure the flow rate of the CSF. After collection of the CSF, the catheter was flushed with sterile saline and the Huber needle was removed. There were occasions that the CSF did not flow from the needle; in some instances the CSF could be obtained by gently aspirating a sample, but other times a sample was unable to be collected. The CSF was cultured for bacteria in order to detect possible contamination. The duration of the models varied from 1-8 months (ongoing), with biweekly cultures totaling 98 cultures in which two animals tested positive with species of streptococcus and staphylococcus. These
two animals were removed from study and the VAP and catheter were removed. The advantage of this system is that it provides a minimally invasive approach to collecting multiple CSF samples from a conscious rhesus for several months.

**P59 Toxicity of h-R3, Anti-Epidermal Growth Factor Receptor Monoclonal Antibody Labeled with 188Os after the Intracerebral Administration in a Model Animal**

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Animal models are of limited use for the study of dosimetry, host immune responses, and normal tissue toxicity. h-R3 is a humanized anti-EGFR antibody, which is overexpressed in glioblastomas. 188Re constitute an ideal radionuclide for imaging and RAIT, but we do not know if 188Os, as 188Re’s daughter, has a local and systemic effect. For this reason we decided to assess the toxicity of stable 188Os once the complete decay of 188Re has occurred, by administering in Sprague Dawley rats the 188Os-labeled h-R3 8 days after the conjugation with radionuclide. Forty rats were distributed into four experimental groups with five animals of each sex in each group. A single 5 µL of neutral solution containing 50 mg of h-R3 labeled initially with 13.25 mCi were administered to each animal. Necropsy and histopathological studies were carried out after completion of the study. All animals gained weight by day 14, indicating that no clinical, biochemical hematological and histopathological disturbances attributable to traumatic insults were observed. MAb h-R3 administered in relatively high doses induced no toxicity. As a noninvasive method of measuring diffusion, it would clearly be preferable in malignant gliomas treatment.

**P60 Hemolytic Escherichia coli Strains Producing Necrotizing CF1 Toxin Are Associated with Enteric and Systemic Diseases in Ferrets**

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Diseases associated with Escherichia coli (E. coli), long prominent in veterinary medicine as important causes of morbidity and mortality, are the subject of renewed interest due to emerging diseases of humans such as hemolytic uremia syndrome (HUS). The ferret (Mustela putorius furo) has a number of E. coli-associated diseases. The most common is gangrenous mastitis, caused by an a-hemolytic E. coli of serotype O:157:H7. In addition, extra-intestinal diseases include pyometra, vaginitis, pyelonephritis, omphalophlebitis and septicemia. Enterotoxigenic E. coli associated with diarrhea and acute death has been described in captive black-footed ferrets. Recently, the ferret has been used as a model of HUS after oral infection with E. coli 0157:H7. A collection of strain of a-hemolytic E. coli were isolated from diarrheic feces and diseased tissue (mammary gland, uterus, brain) of ferrets over a year period. Fourteen of the 15 strains (the most common serotypes being O2H4, O4H-, and O6H-) were positive by specific PCR assay for the presence of necrotizing factor 1, a heat-labile, monomeric 115-kDa protein encoded by a gene on the chromosome of pathogenic E. coli. It acts on Rho GTPases, which participate in the regulation of the actin cytoskeleton in normal cells. CNF1 deaminidates glutamine 63 in RhoA, changing it to glutamate. This change increases stress fibers in the target cell, causing it to enlarge, become syncytial and die. CNF1 producing strains of E. coli are also associated with diarrheal disease in humans as well as dogs, cats, ruminants and pigs. These strains have also been isolated from human extraintestinal disease and septicemic calves. Studying the pathogenesis of this important E. coli associated disease in ferrets will help our understanding of the pathogenic potential of CNF1 producing E. coli strains in humans and other domestic animals.

**P61 Allele-Specific Polymerase Chain Reaction (PCR-SSP) Assays for Genotyping Mutant Mouse Strains**

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Genotyping of the causative gene is a useful technique in the breeding and production of mutant laboratory animals. Several naturally occurring mutant mouse such as SCID (Prkdcscid), diabetic (Leprdb), and obese (Leprdb) possess the mutant gene with a single nucleotide substitution. In this study, we developed quick, easy, multiplex allele specific PCR (PCR-SSP) assays that detect Prkdcscid, Leprdb, and Lepr mutant PCR products extended by wild type allele specific reverse primer (WR) with the counterpart forward primer (F) upstream, and the other forward primer (MF) specific for the mutation with the other counterpart reverse primer (R) downstream. The allele specific PCR products extended by F/WR or MF/R were amplified with common products extended by F/R. Two-color PCR-SSP for genotyping Leprdb and Lepr mutations. PCR with confronting two primer pairs (PCR-CTPP) was performed for genotyping the Ptkα mutation using the wild type allele specific reverse primer (WR) with the counterpart forward primer (F) upstream, and the other forward primer (MF) specific for the mutation with the other counterpart reverse primer (R) downstream. The allele specific PCR products extended by F/WR or MF/R were amplified with common products extended by F/R. Two-color PCR-SSP was performed for genotyping Leprdb and Lepr mutations using forward primers specific for the mutant or wild type alleles labeled with HEX and FITC, respectively, and the common reverse primer. The results showed that three genotypes (homozygotes for mutant and wild, and heterozygote) of the target loci were clearly distinguished by product size or color. The PCR-CTPP and two-color PCR-SSP developed here make it possible to reduce the number of reactions because both alleles can be amplified in the same reaction mixture.

**P63 Detection of Lymphocytic Choriomeningitis Virus by Fluorogenic Nuclease Reverse Transcriptase Polymerase Chain Reaction**

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Lymphocytic choriomeningitis virus (LCMV) induces persistent infections in laboratory mice, is known contaminant of biological materials such as transplantable tumor cell lines, and is of great concern in animal facilities due to its zoonotic poten-
tial. Fluorogenic nuclease reverse transcriptase polymerase chain reaction assays (fnRT-PCR) combine RT-PCR with an internal fluorogenic hybridization probe, thereby potentially enhancing specificity and eliminating post-PCR processing. An fnRT-PCR assay specific for LCMV was therefore developed by targeting primer and probe sequences to a unique region of the LCMV nucleocapsid (NP) gene. The LCMV fnRT-PCR assays detected only LCMV and did not detect other RNA viruses that naturally infect rodents. The fnRT-PCR assays detected as little as 1 pg of LCMV RNA, and displayed comparable sensitivity when directly compared to the mouse antibody production test. The fnRT-PCR assay was also able to detect viral RNA in numerous tissues and in feces and cage swipes collected from experimentally inoculated BALB/c mice, but did not detect any viral RNA in similar samples collected from age- and strain-matched mock infected mice. In conclusion, the LCMV fnRT-PCR assay offers a potentially high-throughput diagnostic assay to detect LCMV in mice and contaminated biological materials.

P64 Detection of Sendai Virus and Pneumonia Virus of Mice by Fluorogenic Nuclease Reverse Transcriptase Polymerase Chain Reaction

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Sendai virus induces acute respiratory disease in laboratory mice and is a common contaminant of biological materials. Pneumonia virus of mice (PVM) also infects the respiratory tract and, like Sendai virus, induces a persistent wasting disease syndrome in immunodeficient mice. Fluorogenic nuclease reverse transcriptase polymerase chain reaction assays (fnRT-PCR) combine RT-PCR with an internal fluorogenic hybridization probe, thereby potentially enhancing specificity and eliminating post-PCR processing. Therefore, fnRT-PCR assays specific for Sendai virus and PVM were developed by targeting primer and probe sequences to unique regions of the Sendai virus nucleocapsid (NP) gene and the PVM attachment (G) gene, respectively. The Sendai virus fnRT-PCR assay was also able to detect viral RNA in numerous tissues and in feces and cage swipes collected from experimentally inoculated BALB/c mice, but did not detect any viral RNA in similar samples collected from age- and strain-matched mock infected mice. In conclusion, the LCMV fnRT-PCR assay offers a potentially high-throughput diagnostic assay to detect LCMV in mice and contaminated biological materials.

P66 Evaluation of Experimental Infections for Further Clinical Trials in Canine Visceral Leishmaniasis

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Visceral leishmaniasis (VL) is a zoonotic disease transmitted by phlebotomine vectors, in which the dog is the principal domestic reservoir. It has been postulated that dog vaccination could be the most efficacious control method, and therefore the establishment of infection protocols is key to clinical trials of vaccine candidates. In this study, 21 female dogs, 4-6 months of age, were inoculated with metacyclic promastigotes of Leishmania chagasi harvested from colonized Lutzomyia longipalpis that were artificially infected. Four different schemes were used: 1)104 parasites i.v.; 2)104 parasites i.d.; 3)105 parasites i.v.; and 4)105 parasites i.d. Seven uninfected were used as controls. Animals were evaluated clinically every 15 days, and both clinically and parasitologically every 2 months. At 6 months p.i., the clinical evolution was variable within each group. Dogs subjected to the high i.v. scheme (105) progressed rapidly towards overt disease, but i.d. infections also produced a variable degree of signs and symptoms (lymphadenopathy, onychogryphosis, dermatitis and charquetia), which interestingly were more frequent with the low inoculum (104). Intravenous inoculations lead to a high frequency of lymph node infection (50-66%), and infectivity to L. longipalpis (2/10 dogs), as opposed to the intradermal schemes in which lymph node involvement was low (20-33%) and no infectivity to vector could be detected. Also, intravenously infected dogs showed the highest antibody production (ELISA, mean O.D. = 1.06) and cellular immune response (2.79 stimulation index), associated with the lowest hematocrit val-
ues (33%). These preliminary results suggested that for short-term studies (<6 months) in which clinical symptoms and infectivity to vectors are used as endpoints, only intravenous infections with \(10^4\) or \(10^5\) metacyclic promastigotes could be used. The intradermal route possibly mimics the natural, protracted evolution of canine VL.

**P67 Genetic Stability of the Integrated Transgene in TgPVR21/IQI mice**

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A transgenic mouse susceptible to poliovirus, TgPVR21, was created in 1991 by introducing the human gene encoding the cellular receptor for poliovirus (PVR) into the ICR mouse genome. TgPVR21/IQI was established by backcrossing TgPVR21 to the IQI inbred strain to make a genetically homogeneous population and has been developed as a novel animal model in place of monkeys for evaluating neurovirulence of oral poliovirus vaccine (OPV). In 2000, a new standardized neurovirulence test of OPV using TgPVR21/IQI mice was approved by the World Health Organization. Instability of the transgene directly affects the experimental results in such a safety assessment. Therefore, it is important to assure the stability of the transgene by checking its structure, expression and function periodically to provide genetic engineered mice as an animal model. In this study, we evaluated the genetic stability of the integrated transgene in TgPVR21/IQI mice by investigating its structure and expression at past N3 and present N20 generations. FISH analysis revealed that the integrated PVR gene was located at chromosome 13B3 region in both generations. Southern blot analysis did not show any differences in the hybridized band patterns between the two generations and their bands were the same size as those reported in 1991. Expression of the transgene in the brain was observed in both generations by Northern blot analysis. Three mRNA isoforms, PVR alpha, PVR beta and PVR gamma, were detected by RT-PCR assay in the brain, kidney and intestine obtained from N3 and N20 trangenic mice. Nucleotide sequences of PVR alpha gene that encodes the functional receptor molecule were determined by RT-PCR-direct sequencing and perfectly matched those reported previously. These results suggested that the transgene of TgPVR21/IQI mice is stably transmitted over generations. We also discussed the new concept of “transgene monitoring” that assures the quality of transgenic animals.

**P68 Host Protease Activity Is Upregulated in Canine Periodontitis**

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Background: Periodontitis is characterized by extensive destruction of the gingival tissues and associated supporting structures of the teeth. Although the pathogenesis of the various forms of this disease is not completely understood, host-derived proteases are believed to have an important role. In this study, we analyzed tissue samples from chronic adult dogs with or without periodontitis to assess the levels of specific proteases and determine the effect of pH and tetracycline on their activity.

Methods: Gingival tissue samples were obtained from eight adults with chronic periodontitis or periodontally healthy conditions in the Veterinary Hospital at the University of Pennsylvania (n = 4). Tissue extracts were prepared and analyzed for protease activity by zymography and Western blotting. The possibility of protease derived from the bacteria isolated from the dog gingival tissue such as *Porphyromonas gingivalis*, *Bacteroides forsythus*, and *Actinobacillus actinomycetemcomitans* was also evaluated with the same assays under the same conditions.

Results: Matrix metalloprotease activity from clinically normal and diseased tissue was observed at pH 8, and was dramatically upregulated in diseased tissue. Latent matrix metalloproteinase (MMP-2 and MMP-9) were expressed in all samples examined, while active MMP-2, MMP-9 was detected only in tissue obtained from dogs with clinical disease. The MMP activities were differentially inhibited by Doxycycline. At pH 6, a protease with a molecular weight approximately 38 Kda was observed in diseased samples. This enzymatic activity was inhibited by phenylmethylsulfonyl fluoride (PMSF), suggesting it is a serine protease. Protease activity was not present in bacteria or their conditioned medium.

Conclusions: The results of the current study demonstrate the potential role of host-derived proteases, possibly inflammatory cell derived in part, in the pathogenesis of canine chronic periodontitis. The results also indicate that the proteases present in dog tissue samples were host-derived, rather than derived from bacteria in the site. Specifically, the results indicate that activated MMP-2, MMP-9 and a 38 Kda serine protease may be involved in tissue destruction associated with this form of periodontal disease, and therefore, inhibiting proteinase activity may improve the treatment of canine periodontal disease. The study also suggests that tissue pH influence protease activity at sites of diseases and that tetracycline or its derivatives (doxycycline) are therapeutic reagents of high potential value in this disease.

**P69 Influence of Sex Hormones on Physiological Stress and Cerebral DNA Damage in Elderly Male Brown-Norway Rats**

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Data from our laboratory suggest that the testis contributes to the steady state level of DNA damage within the brain of elderly dogs. The concentration of corticosterone in feces provides a non-invasive measure of physiological stress in rodents. The purpose of this study was to evaluate the possible association between physiological stress and the DNA damage-sparing effect of castration within the brain. Elderly (20-month-old) male Brown-Norway rats were housed individually and randomly assigned to groups: surgical castration (n = 5); surgical castration plus supra-physiologic testosterone replacement (n = 5); and sham-operated controls (n = 7). Serum testosterone was measured by RIA at 2, 6, and 10 weeks. Fecal samples were dried and assayed for corticosterone by HPLC, and measurements at 2, 4, 6, 8, and 10 weeks were used to calculate average fecal corticosterone for each rat. After rats were euthanized at 10 weeks, Fpg alkaline comet assay was used to measure oxidative DNA damage in the cerebrum. Castrated rats had the lowest fecal corticosterone levels (mean ± SD = 714 ± 169 ng/g dry feces). Testosterone-treated rats had the highest fecal corticosterone levels (mean ± SD = 1687 ± 488 ng/g). Mean values in both the castrated and testosterone-treated groups were significantly different from sham-operated intact controls (P = 0.04 and P = 0.004, respectively). Overall, there was a significant correlation between serum testosterone and cerebral oxidative...
DNA damage ($r^2 = 0.44; P = 0.004$). Similarly, there was a strong positive correlation between serum testosterone and fecal corticosterone concentrations ($r^2 = 0.53; P = 0.0006$). Fecal corticosterone levels were also strongly correlated with cerebral oxidative DNA damage ($r^2 = 0.56, P = 0.0004$). These results suggest that modulation of testosterone significantly influences the amount of physiological stress and the extent of oxidative damage within the brain of elderly male rats. Additional studies are needed to further define the association between sex hormones, the pituitary-adrenal axis, and DNA damage within the mammalian brain.

P70 Neutralization of Interferon-γ Decreases Typhlitis Associated with *Helicobacter hepaticus* in A/JCr Mice

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*Helicobacter hepaticus* infection causes typhlitis and colitis in immunocompromised mice and susceptible strains of immunocompetent mice (e.g., A/JCr). This animal model provides the unique opportunity to study inflammatory bowel disease triggered by a bacterial antigen in an immunocompetent host. Neutralization of IFN-γ in interleukin-10-deficient mice has been shown to significantly decrease cecal scores when mice are treated before inflammation is established but not when mice are treated after inflammation is present. This suggests that IFN-γ is important in initiation but not necessary for maintenance of inflammation in the absence of IL-10. In this study, immunocompetent *H. hepaticus*-infected A/J mice were treated for two months with a neutralizing antibody to IFN-γ to further define the role of this cytokine in an immunocompetent host. Mice were treated at two different timepoints following experimental inoculation: 1 month, a timepoint preceding development of cecal inflammation; and 3 months, a timepoint when typhlitis is established. At both timepoints, antibody-treated mice had a significantly lower median cecal score ($P = 0.007$ and $P = 0.041$). These findings in conjunction with those obtained from IL-10 knockout mice indicated that established bacterial-induced inflammation may be abrogated by neutralization of IFN-γ, but only in the presence of IL-10.

P71 Physiological Melatonin Levels, Omega-3 Fatty Acids, and Conjugated Linoleic Acid Inhibit Fatty Acid Transport in Rat Skeletal Muscle

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The pineal neurohormone melatonin (MLT), secreted in mammals primarily at night, eicosapentaenoic acid (EPA), and conjugated linoleic acid (CLA) have established regulatory roles in cancer growth. Recent investigations in our laboratory have demonstrated that these agents suppress fatty acid (FA) uptake by tumors, as well as inhibit FA transport in several sub-types of white adipose tissue via inhibitory G protein-coupled receptor mechanisms. We employed a surgical technique for catheterizing the femoral vein in Sprague Dawley rats that allows for rapid blood collection from the entire hind limb. Arterial blood was collected from the carotid artery, and nutrient uptake was calculated from arteriovenous differences. In this study we tested the hypothesis that physiological MLT levels, EPA or CLA, injected intravenously, inhibit FA uptake in skeletal muscle in vivo. Fatty acid oxidation is a major source of energy in skeletal muscle. Total FA uptake by the hind limbs of fed control rats (n = 12), and animals (n = 3 per treatment group) injected intravenously with MLT (1 nM), EPA (0.5 mM), or CLA (10t, 12c isomer, 0.09 mM) was $6.07 = 1.57$, $-1.39 = 0.61$, $0.65 = 0.98$, and $0.04 = 0.80$ mg/min/g tissue wet weight, respectively. Arterial/venous values for pH (7.43 ± 0.4/7.29 ± 0.08), pO2 (155.6 ± 3.2/35.6 ± 10.2 mm Hg) and pCO2 (29.7 ± 10.9/57.0 ± 7.5 mm Hg), and venous blood flow rates ($0.277 = 0.014$ ml/min) were recorded and remained constant during the course of each experiment. To our knowledge, this is the first demonstration that physiological MLT levels, EPA, and CLA (10t,12c) inhibit FA transport in skeletal muscle, a major tissue in vertebrates. These investigations suggest a novel role for MLT, omega-3 FAs, and CLA in the regulation of fatty acid transport and fat metabolism in skeletal muscle.

P72 Substrain Differences in the Immune Response to *Bordetella bronchiseptica* Infections in C3H Mice Due to TLR 4 Mutation

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Many species of laboratory animals, including mice, rats, guinea pigs, and rabbits, are naturally susceptible to chronic *B. bronchiseptica* infections, as the bacteria are not readily cleared from the respiratory tract. In order to understand defense mechanisms important in bacterial clearance, differences in the ability of C3H/HeN (TLR 4 intact) and C3H/HeJ (TLR 4 mutant) to clear *B. bronchiseptica* infections were examined. TLR 4 may be important in the immune response to *B. bronchiseptica*, as it is a major receptor for lipopolysaccharide and triggers signaling cascades that mediate phagocytic inflammatory cell responses and cytokine expression. Age-matched C3H/HeJ and C3H/HeN mice were intranasally challenged with $5 \times 10^8$ CFU of *B. bronchiseptica* and examined at 1 and 3 days postinoculation. The C3H/HeJ mice were colonized by *B. bronchiseptica* at 10–10,000-fold higher levels than their C3H/HeN counterparts. Additionally, bronchialveolar lavage fluid from C3H/HeJ mice had fourfold fewer neutrophils than lavage fluid from the C3H/HeN mice. Lung lesions in C3H/HeJ mice were more severe as compared to C3H/HeN mice. When C3H/HeN mice were injected with immune serum at the time of infection, they had significantly less CFU in the lungs and trachea as compared to untreated C3H/HeN mice. However, by the same method, adoptive transfer of antibodies to the TLR 4 deficient C3H/HeJ mice had no effect on the number of CFU in the lungs and trachea. Lung lesions in C3H/HeN mice decreased in severity between days 1 and 3 upon treatment with immune serum, whereas lung lesions increased in severity in treated C3H/HeJ mice. These results show that these two substrains of C3H mice differ substantially in their ability to clear *B. bronchiseptica*, and that TLR 4 is important in controlling bacterial numbers, aids in neutrophil recruitment, helps minimize lung pathology, and is required for antibody-mediated clearance of *B. bronchiseptica* from the lower respiratory tract of mice.

P73 Susceptibility and Pathogenesis of Vaccinated American Crows and Sandhill Cranes to West Nile Virus

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West Nile Virus (WNV) was recently introduced into the United States in the vicinity of New York City. In this location the virus

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has killed a large number of birds, especially, but not limited to, crows. Infected American Crows (Corvus brachyrhynchos) were likely responsible for the geographic expansion of WNV into adjoining counties and states. Because of the virus spreading into the Southern and Midwest states, Greater Sandhill Cranes (Grus canadensis) and other crane species are at risk.

All crows were captured locally in Wisconsin and housed in cages for two experiments and in open-room, free-flying arrangement for four experiments. The total number of crows used in experiments over the 2-year period was 100: 49 were needle-inoculated subcutaneously with WNV (NY99 Strain) virus and 47 died with in 3-8 days following inoculation, 3 were treated orally and all died, and 42 were treated differently (uninoculated controls or vaccinated with two different vaccines and challenged with WNV) with mixed mortality patterns. Eleven Greater Sandhill Cranes of mixed age (1-5 years) and sex were divided into two groups: 6 unvaccinated birds and 5 vaccinated birds. The vaccinated cranes were inoculated with a newly developed commercially available Fort Dodge Killed-virus WNV equine vaccine in the left pectoral muscle with a 0.5 ml dose on days 0, 21, and 28. Unvaccinated birds were inoculated with 0.5ml of sterile saline. After receiving an initial dose of vaccine and 2 boosters, cranes were inoculated subcutaneously with 0.1 ml of 5,000 pfu WNV isolate from American Crow in Suffolk County, New York. 3 ml of blood samples were collected from the jugular vein the first 8 days postchallenge and on days 10, 14, 21, and 42. Blood samples were used to check for evidence of viremia and for CBC and serum chemistry. All cranes survived through study with mixed results on virus isolation and neutralizing antibody.

P74 The Effects of Conditioning on the Heart Rate of the Conscious Experimental Mouse

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Background: Traditional measurements of heart rate (HR) on conscious mice are usually performed while the animal is under restraint, or tethered to physiological monitors. Conditioning (i.e., getting animals accustomed to handling during conscious experiments) has been proposed as a method to lessen the stressor effect of restraint on the experimental mouse. In this study, we sought to determine if conditioning has an effect on HR, and if this effect is cumulative over time.

Methods: Six normal adult CD-1 mice were instrumented with miniaturized ECG radio-frequency telemetry implants and allowed to recover for ten days prior to study initiation. ECG was continuously recorded for 3h in the following sequence: (i) 15 min. prior to restraint (baseline), (ii) 45 min. of handling and restraint (conditioning), and (iii) 2 h after release and replacement in their cages (postconditioning). This procedure was repeated daily for 10 days. The mean HR for each day was then calculated from 5-sec. sampling intervals and plotted to determine the trend. This data was then compared with control (no conditioning) recordings from corresponding time intervals obtained on each mouse.

Results: All results are reported as mean ± S.D. A plot of HR over 15-minute intervals is seen in the graph below. When compared to corresponding control intervals, an initial increase in HR was observed in the conditioned animals (599 ± 51.5 versus 773 ± 21.5). This increase in HR persisted throughout the conditioning period. During the postconditioning period, a continued decrease in HR was observed, reaching baseline levels after 1 h. No statistical differences in HR were seen throughout the 10 days.

Conclusion: Handling during conditioning significantly increases baseline heart rate, suggesting a stress response. Prolonged conditioning does not appear to reduce this response, nor accelerate return to normal heart rate levels in conscious mice.

P75 Transgene Stability and Features of rasH2 Transgenic Mice

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The transgenic mouse rasH2 line, in which the mouse carries the human c-Ha-ras gene under the control of its own enhancer and promoter, has been proposed as one of the alternative short-term models for carcinogenicity testing. To study this application, we produced a genetically homogeneous population as C57BL/6Jic-TgN(RASH2) (Tg-rasH2) by continuous backcrossing. In this study, we examined (1) transgene stability between different generations and during large-scale propagation, and (2) detailed transgene architecture of the integrated human c-Ha-ras gene. FISH analysis showed that the integrated human c-Ha-ras gene was stably located on chromosome 15E3 in Tg-rasH2 mice at generation numbers (N) 15 and 20. Southern and Northern blot analysis did not show any differences in the hybridized band pattern in each generation. Furthermore, in Southern blot analysis of over 1,000 Tg-rasH2 mice, we did not find any differences among individual DNA samples. By detailed Southern blot analyses, we proved that the Tg-rasH2 mouse contains three copies of human c-Ha-ras gene arrayed in a head-to-tail configuration. We also determined the nucleotide sequence of the transgene in the Tg-rasH2 mouse at N20 and confirmed that the sequence of the coding region was perfectly matched with human c-Ha-ras cDNA. We determined the structure of genome/transgene junctions, revealing that integration of the microinjected human c-Ha-ras gene into the mouse host genome resulted in a 1,820-bp deletion in the rasH2 line. The deleted sequence did not have any sequence homologies with known functional genes. We demonstrated that the integrated transgene in Tg-rasH2 mice was stably transmitted over several generations and during large-scale propagation. However, we believe that checking of the genotype and phenotype is required at regular intervals in Tg-rasH2 mice used for carcinogenicity testing because possible contamination with nonresponder mutants in the foundation colony would affect the reliability of carcinogenicity test results.

P76 Epidemiological Survey of Wild Atlantic Bottlenose Dolphins (Tursiops truncatus) for Helicobacter spp. Infection by PCR, Southern Blot and ELISA

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Novel Helicobacter spp. have been isolated from wild and captive Atlantic bottlenose dolphins, Tursiops truncatus. Clinical signs in infected captive dolphins have been either subclinical or have included chronic regurgitation, intermittent inappetence, weight loss, lethargy and in some cases associated with gastric lesions. Our previous studies suggested that helicobacter infection may play a role in the development of...
gastritis in these dolphins. Feces and serum were collected from 14 wild Atlantic bottlenose dolphins by Sarasota Dolphin Research Program in Florida as part of a wildlife survey. Fecal samples were tested using a genus-specific PCR assay and Southern blot for *Helicobacter* sp. Serum was tested by ELISA for Helicobacter-specific IgG directed against an outer membrane antigen preparation of the *Helicobacter* sp. isolated from feces by culture. Five of 14 dolphin fecal samples were positive for *Helicobacter* sp. by both PCR and Southern blot, and two additional samples were PCR-negative but Southern blot positive. Two of these 7 dolphins testing positive by PCR or Southern were seropositive by ELISA and the remaining 5 were seronegative. All dolphins that tested negative by PCR or Southern Blot were also seronegative. These results indicate a 50% incidence of *Helicobacter* spp. in these 14 wild dolphins and suggest that PCR and Southern blot are more sensitive compared to the ELISA, which was 100% specific but only had a 29% sensitivity. Further optimization of the ELISA assay is warranted because it would be a useful non invasive screening test in diagnostic evaluations of dolphins presenting with signs of gastrointestinal disease.

**P77 Conjunctivitis and Ocular Discharge in a Pig (Sus scrofa domestica)**

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A young adult female Yorkshire-Duroc cross pig, with one blue eye (left) and one brown eye (right), presented for mucopurulent ocular discharge and conjunctivitis of the blue eye. This pig had a right carotid-jugular fistula surgery 5 months prior to presentation as part of an experimental protocol. No other abnormalities were noted on physical examination. Differential diagnoses included bacterial or viral infection, corneal ulcer, keratoconjunctivitis sicca, and dacryocystitis. A Schirmer test and fluorescein stain were normal. Conjunctival cytology demonstrated many neutrophils and macrophages admixed with mucin and mites with elongated bodies and anteriorly-located grouped appendages (*Demodex phylloides*). No mites were observed from the swab from the unaffected brown eye. Culture results revealed growth of coagulase negative staphylococcus and were negative for Chlamydia. Treatment included 0.3 mg/kg Ivermectin, which was repeated 2 weeks later, and twice-daily triple antibiotic ophthalmic ointment. The affected eye improved after one week of the treatment, but after the second dosage of ivermectin the eye became enophthalmic with ptosis and possible miosis, indicative of Horner’s Syndrome. It also had medial strabismus on the same eye. CBC and serum chemistries were within normal limits. At the end of the study Demodex parasites were identified histologically within the follicles and ducts of ciliary sebaceous glands of the eyelids and granulomatous inflammation associated with folliculitis and follicle rupture on the affected eye. A full necropsy was mistakenly not done, but the complex of Horner’s Syndrome and strabismus suggested an intracranial cause. To our knowledge, Demodex has not previously been described in association with conjunctivitis in the pig. Because the brown eye remained unaffected by either disease condition, we suggest that the lack of pigmentation in the eye of this pig might have predisposed the animal to the conditions described in this report and should be selected against.

**P78 Innovative Wound Care System for Non-Healing Wounds in Nonhuman Primates**

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Wound care management in nonhuman primates often presents a challenge to the veterinary care staff that is unique to laboratory animal care due to their overt tendency to pick at their wounds, resulting in delayed wound healing. An optimal system would be one that affords protection to the site of injury, allows free movement of the animal, does not hinder the healing rate, is safe to the animal, indestructible and is low maintenance for the care staff. We devised a system using PolyMem Dressing (Ferris Mfg.) and a jacketed bandage approach to treat a non-healing fight wound. By using this system, we were able to heal the wound in a short period of time with minimal discomfort to the animal. PolyMem is an innovative wound dressing that has been used in the human market for fourteen years, especially useful in non-healing diabetic ulcers. It contains a cleanser, moisturizer, and an absorbing agent that creates an optimal wound-healing environment, making it unnecessary to clean the wound between bandage changes. To protect the dressing, the arm was bandaged, wrapped to the body and a primate jacket was used to protect the bandage. Using the PolyMem dressing in conjunction with the jacketed bandage allowed complete healing of an otherwise non-healing wound.

**P79 The Prevalence of Giardia in a Population of Dogs Housed at a Research Facility**

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*Giardia* is an enteric protozoan parasite known to infect a broad range of hosts, including many laboratory species. The parasite is commonly encountered in dogs and is the most frequently identified intestinal parasite of humans. In the past year, two cases of diarrhea in research dogs due to giardiasis were diagnosed within our facility. These cases revealed the presence of *Giardia* within the facility and up to this point our health-monitoring program had not addressed this organism. The objective of the study described here was to determine the prevalence and spatial distribution of *Giardia* infections in dogs within the facility and to derive a control program that would minimize the number of infections. *Giardia* antigen-ELISA tests were performed on feces from all dogs (107 mixed breeds) housed on both floors of the facility and the prevalence of *Giardia* was determined to be 11% (12 dogs). All dogs were asymptomatic at the time of testing. In addition, there was a higher prevalence of *Giardia* infections in dogs housed on the second floor that may be a reflection of room design. Management strategies involve treating infected dogs and all other dogs housed in the same room for 3 days with fenbendazole per os, 50 mg/kg q 24 h. On the last day of treatment, dogs are bathed and returned to runs that have been disinfected with a quaternary ammonium compound. Dogs are retested for *Giardia* infection 3 weeks after the last day of treatment. The *Giardia* antigen-ELISA test was found to be a cost-effective method for rapidly screening a large number of dogs. However, the health risk of asymptomatic antigen-positive dogs for humans and other animals is not well understood. Until proven otherwise it is prudent to assume that such dogs are a potential zoonotic threat.
P80 An Outbreak of Murine Respiratory Micoplasmosis Associated with SDAV Infection in a Rat Colony from an Experimental Unit at State University of Campinas, Brazil

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Mycoplasma pulmonis infection and disease are common in conventionally reared rats and mice at Brazilian animal facilities. The disease symptoms vary greatly in expression because of environmental conditions, concurrent infections of the respiratory tract with others pathogens, strain virulence and host factors that influence host-parasite relationship. One rat colony from an experimental unit at State University of Campinas showed signals of a MRM and SDAV infection outbreak. The clinical symptoms observed were accumulations of porphyrin around eyes and external nares, megaloglobus, snuffling, polyneuropathy, hunched posture and weight loss with death after one week. A sample of 10 animals 4-8 weeks old showing clinical symptoms was selected from the colony and submitted at gross necropsy for microbiological monitoring. At necropsy 30% of the submandibular salivary glands was enlarged and blanched and 100% of lungs showed different degrees of lesions as reddish, hemorrhagic, mucous, bronchiectasis and pulmonary abscesses. Sera samples were obtained for serological screening. Small slices of lung tissue were aseptically collected and grown in sheep blood agar, supplemented PPLO broth and PPLO agar at 37°C under atmosphere with 5% CO2 gas. Characteristic microscopic Mycoplasma sp. colony growth on PPLO agar after sub-culture from PPLO broth was identified as M. pulmonis by biochemical tests and anti-serum inhibition growth test. Serology was 100% strongly positive for M. pulmonis in ELISA and IFA tests and for rat Coronavirus (SDAV) by Indirect Immunofluorescence method using MHV as antigen. The possible causes of this outbreak were the fact that this unit has kept animals in bad environmental conditions under a non-sanitary barrier, and in the past animals were obtained from different breeders. All experimental procedures were disrupted, animals were euthanized and a well-colony management program was established. The economic and scientific wastes point to the need for a sustained effort with the aim to improve the quality of laboratory animal conditions at Brazilian experimental facilities.

P81 Analgesic Potential of Carprofen in Mice

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The ability of diluted carprofen to produce analgesia in two strains of mice was evaluated using the hot plate test. Sixty CD-1 and 60 C57BL/6 male mice, 6 weeks of age, were divided into groups of 20. Each group was used to evaluate carprofen (supplied as a 50 mg/ml micelle solution) at a dose of 5 mg/kg, 10 mg/kg or 20 mg/kg. The latency to respond to the hot plate (maintained at a temperature of 55 ± 0.1°C) was measured and recorded in seconds. A response was considered a hind paw lick, shake, or tap. A cut-off time of 35 sec. was used to prevent tissue damage. After obtaining three baseline measurements on each animal, carprofen diluted with saline was administered by subcutaneous injection. Measurements were taken at 15, 30, 60, 90, 120, 150, 180, 240, 300, 360, 420, 480, and 540 min. after injection. For CD-1 mice, results indicate the low dose carprofen (5 mg/kg) had no significant effect on latency at any timepoint. The mid dose (10 mg/kg) increased latency time at two timepoints, 240 and 300 min. after injection. The high dose (20 mg/kg) increased the latency time at all timepoints from 90-180 min. For C57BL/6 mice, a variable latency response was seen in mice given low dose carprofen (5 mg/kg). Latency times increased significantly at 120 to 180 min. and again from 360 to 540, but were near baseline at the 240-300 timepoints. In contrast, carprofen at the mid dose (10 mg/kg) significantly increased latency times at all timepoints from 60 to 340 min. postinjection. A variable latency response was also seen in the C57BL/6 mice given the high dose (20 mg/kg), resulting in increased latency times from 120 to 300 min. postinjection and at the 360-min. timepoint. Although there was variability within the data, increased latency times on the hot plate test suggests diluted carprofen produced analgesia at the higher doses in both strains tested. Carprofen is formulated as a micelle solution. The variability observed may be due to the dilution affecting the micelle solution and thus the analgesic properties of carprofen. Further studies are underway to evaluate this variability. Carprofen has been recommended at a dose of 5 mg/kg in mice. Our data suggests that 10 mg/kg or 20 mg/kg may be a more effective analgesic dose.

P82 Assessment of Retinal Degeneration in Outbred Albino Mice

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Despite the genetic diversity that breeders attempt to maintain in outbred populations, certain genetic constitutions develop that provide resultant sensitivities of which both breeders and scientists are unaware. Increased sensitivity to light in a laboratory animal can confound the interpretation of ophthalmic data generated from a safety evaluation study. Retinal degeneration (RD) was observed to occur in outbred albino mice (HsdICR.CD1). Such a pathologic manifestation has been reported to be associated with an autosomal recessive gene rd, retinal degeneration. In an effort to evaluate the genetic basis for the observation of RD in HsdICR.CD1 mice, a study was conducted comparing the light sensitivity of the following stocks/strains of mice: Hsd:ICR(CD-1); Hsd:NSA(CF-1); HsdWin:CFW-1; Crl:CD-1(1CR)BR; CrI:CF-1BR; and C57BL/6-Tyr-/-BR. FVB/NcrBR was used as a positive control since it has been established that these animals carry an rd gene. C57BL/6-Tyr-/- was used as a negative control since these animals have been reported to be resistant to retinal degeneration. Mice were observed over a 12-week period of time. Average room and in-cage illumination were determined. Hematology and serum clinical chemistry revealed no abnormal findings. Histopathologic evaluation revealed clear differences. Affected stocks/strains did not exhibit sex-related differences, except for Hsd:ICR(CD-1) mice, in which retinal atrophy was almost twice as common in males as females. Incidences of retinal atrophy for the various stock/strains were: Hsd:ICR(CD-1) (43.3%); CrI:CF-1BR (3.0%); and FVB/NcrBR (98.3%); Tacs:ICR:H:Ha (17%); Tac(SW) (80%) and FVB/NcrBR (100%). No retinal atrophy was observed in the following strains: Hsd:NSA(CF-1); HsdWin:CFW-1; Crl:CD-1(1CR)BR; and C57BL/6-Tyr-/-.
P83 Co-Infection of Laboratory Rats with Mycoplasma pulmonis and Chlamydia pneumoniae

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Background and purpose: Routine examinations of the conventional outbred Wistar rats, carried out in our laboratory, showed an increase in the serum levels of the enzymes alkaline phosphatase (AP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and urea, which are cellular function indicators. Autopsy of these rats demonstrated the presence of two bacteria, Chlamydia pneumoniae (C. pneumoniae) and Mycoplasma pulmonis (M. pulmonis), in many organs, suggesting that the serum alterations could be due to such co-infection.

Methods: Sections from heart, lung, liver, intestines, spleen and kidney of the rats were stained with haematoxylin and eosin technique, examined under a transmission electronic microscope and submitted to immunoperoxidase and in situ hybridization reactions.

Results: Damage to cellular structures was compatible with the serum level alterations of AST, ALT, AP and urea. Electron microscopy and specific reactions evidenced C. pneumoniae and M. pulmonis in lung, liver, spleen, heart and kidneys.

Conclusion: Natural C. pneumoniae infection, associated with M. pulmonis, may causes deep changes in organs’ structures, which reflect in the serum levels of cellular function indicators.

P84 Hematologic and Serum Biochemical Reference Values of Vervet Monkeys (Cercopithecus aethiops sабaeus) by Age and Sex

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Hematologic and serum biochemical values are of great importance in assessing an animal’s health status. Normal reference ranges for vervet monkeys (Cercopithecus aethiops sабaeus) have seldom been reported, making it difficult for clinicians to interpret blood values. The purpose of the study was to determine a normal reference range interval for hematologic and serum biochemical values in vervets based on age and sex. Blood samples were collected from 140 healthy vervet monkeys, 60 females and 80 males, group housed in outdoor enclosures at the University of California at Los Angeles—Vervet Research Colony. Male and female data were displayed separately within five age categories (1 to 2 years, > 2 to 3 years, > 3 to 4 years, > 5 to 6 years, and > 6 years), and the effects of sex and age on these values were examined statistically. Multivariate analyses of variance were performed on 37 hematologic and serum biochemical values, with P-values ≤ 0.05 considered significant. Significant age-related differences were observed for red blood cell count, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, alkaline phosphatase, albumin, globulin, direct bilirubin, blood urea nitrogen, creatinine, glucose, calcium, phosphorus, total carbon dioxide, potassium, albumin/globulin ratio, blood urea nitrogen/creatinine ratio, and sodium/potassium ratio values. An age-sex interaction accounted for significant differences in the values for red blood cell count, hemoglobin, hematocrit, alkaline phosphatase, cholesterol, calcium, total carbon dioxide, chloride, potassium, sodium, and sodium/potassium ratio. This study provided normal hematologic and biochemical values from clinically healthy vervet monkeys and demonstrated that age and sex influenced those values.

P85 Hematological and Serum Chemistry Values for Wild Caught Thirteen-Lined Ground Squirrels (Spermophilus tridecemlineatus)

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Complete blood count and serum chemistry values are presented for wild caught thirteen-lined ground squirrels (Spermophilus tridecemlineatus). Red blood cells indices were slightly increased compared to previously published data. Serum cholesterol and glucose levels were found to be elevated at 325 mg/dl and 321 mg/dl, respectively. Unusually high serum potassium levels (9.4 mEq/L) were detected in the sampled animals. Statistically significant sex differences were not observed for the parameters measured except for creatine kinase (CK), blood urea nitrogen (BUN), and serum chloride, which may be attributed to the small number of males (n = 5) compared to the number of females (n = 47), as well as the presence of extreme values in both sexes. CK was significantly higher in males than females (P < 0.0001), whereas BUN and chloride were higher in females (P < 0.05). Complete blood count and serum chemistry values for the animals sampled presumably represent those normally seen in the thirteen-lined ground squirrels just prior to hibernation, as the samples were obtained in September. To our knowledge this is the first report of a comprehensive serum chemistry panel for this species. As the thirteen-lined ground squirrel is increasingly being used as an experimental animal, the base line data reported here could be useful for future investigations. These data, however, should be interpreted with caution as values correspond to mid- and late September, just prior to hibernation, and may be not representative for other seasons of the year.

P86 Monitoring the Effects of Repeated Blood Sampling on ECG, Body Temperature, and Blood Pressure Using Telemetry Devices

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In our in vivo rodent experiments, we typically limit acute blood drawing to >20% of the total blood volume per animal. This limit was established to avoid the effects of low blood volume from interfering in our experiments, as well as causing the animal undue stress. In these experiments, we used telemetric monitoring of animals during repeated blood drawing to observe systemic effects of blood sampling on the animal up to and beyond this threshold. Use of wireless telemetry devices in these studies allows for measurement of ECG, body temperature, and blood pressure. Telemetric monitoring devices and subcutaneous jugular vein ports were surgically implanted into each of three normal male Sprague Dawley rats 11 to 12 weeks of age and weighing between 256 to 315 g. After a seven-day recovery period, serial blood sampling studies were conducted on each rat while collecting telemetry data. On the day of sampling, a line fitted with a needle was inserted into the subcutaneous port using brief general anesthesia. A four-way valve was tethered to the needle and placed on top of the cage for repeated sampling. At intervals of 15 min., 0.5ml of blood was
collected from each animal up to one hour, and every 30 min, thereafter, to reach at least 40% of the animal’s total blood volume withdrawn. ECG effects including declines in Heart Rate and QA Interval were seen after 30-40% of the total blood volume was withdrawn. Mean blood pressure showed declines after 20 to 30% withdrawal of total blood volume. Body temperature showed declines after 20% withdrawn. Using this cutting-edge technology to observe these effects provides quantitative assessments that support our policies on limiting blood drawing to 20% of total blood volume. In this way we will protect the integrity of experimental data from systemic stresses caused by low blood volume.

**P87 Relationship of Creatinine Kinase, Lactate Dehydrogenase and Aspartate Aminotransferase Serum Levels to Cardiomyopathy in the Owl Monkey (Aotus vociferans)**

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The purpose of this study was 1) to determine reference values for serum creatinine kinase (CK), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) in captive-born and wild-caught owl monkeys (Aotus vociferans) and 2) to assess the usefulness of these values for the diagnosis of myocardial disease. Serum samples were taken from 12 captive-born and 12 wild-caught owl monkeys housed at the Center for Reproduction and Conservation of Nonhuman Primates in Iquitos, Peru. Urine samples were also collected for semiquantitative testing and tissue samples for histologic and ultrastructural examination were taken from two animals in the wild-caught group that died from severe cardiac disease. Though no significant statistical differences were noted between groups when comparing mean CK, LDH and AST values, mean CK value in wild-caught monkeys was higher (181.50 IU/l) than in captive-born animals (108.33 IU/l). Monkeys with proteinuria, however showed marked statistical differences in CK mean value (192.06) when compared to those without proteinuria (40.20 IU/l, p = 0.021). Seven monkeys (29%), three captive-born (25%), and four wild-caught (33%) had CK/AST ratios that reflected myocardial infaracts (CK/AST ratio 2.9). Gross examination revealed concentric hypertrophy of the left ventricle and slightly contracted kidneys. Histologically, the myocardium had contraction band necrosis and was fibrotic. The muscular layer of the coronary arterioles of the heart, medium-sized arteries and afferent glomerular arterioles of the kidneys were hyperplastic and hypertrophied. Renal glomeruli were hypercellular and had increased mesangial matrix and Bowman’s capsule was fibrotic. Similar changes were noted ultrastructurally. These findings suggest that CK, AST and LDH along with a urinalysis provide a reliable method for diagnosing cardiomyopathies in owl monkeys and strongly suggest that owl monkeys suffer from arterial hypertension and chronic myocardial infarcts.

**P88 The Effects of Stillbirths and Miscarriages on Future Reproductive Performances in the Baboon (Papio spp.)**

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In order to maximize breeding efficiency in a baboon facility it is important to retain animals that are reproductively sound. These are baboons that will deliver a healthy infant, nurse the infant to weaning, exhibit postpartum estrus, conceive and experience a subsequent uneventful parturition followed by a repeat of the above scenario. However, all animals do not exhibit these desired characteristics. For example, there are animals that experience simple (i.e., no medical complications) miscarriages or stillbirths either spontaneously or as a result of experimental procedures. We chose to investigate the effects of miscarriages and stillbirths on baboon future reproductive performances. Previously, we reported that the return to cyclicity (first fertile estrus) and time from that estrus to conception for simple miscarriage and stillbirth animals are statistically similar to those times for normal control animals. Therefore, we concluded that simple stillbirths and miscarriages had no effects on the baboons’ ability to reinitiate estrus and conceive. At that time we had insufficient data to evaluate the outcome of conceptions subsequent to a simple miscarriage or stillbirth. At this time we can report that there is a significant probability that animals suffering either a stillbirth or a miscarriage will experience a recurrence of these conditions during the subsequent pregnancy. For stillbirth animals upon rebreeding two of six animals (33.3%) suffered a second stillbirth, and for miscarriage animals 3 of 12 (25%) suffered a miscarriage following rebreeding. In our facility the spontaneous stillbirth rate is approximately 7% and the miscarriage rate is approximately 11%.

**P89 The Sedative and Behavioral Effects of Nalbuphine in Dogs**

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We compared the degree of sedation, and frequency and intensity of adverse behaviors in dogs associated with nalbuphine when combined with acepromazine or xylazine compared to acepromazine or xylazine alone. Twenty-four dogs (13 females, 11 males) undergoing routine ovariohysterectomy or castration were randomly assigned to one of four groups. Group 1 received nalbuphine 0.5 mg/kg and xylazine 0.5 mg/kg subcutaneously (s.c.). Group 2 received xylazine 0.5 mg/kg s.c. Group 3 received nalbuphine 0.5 mg/kg s.c. and acepromazine 0.05 mg/kg s.c. Group 4 received acepromazine 0.05 mg/kg s.c. All dogs received glycopyrrolate 0.01 mg/kg s.c. Preoperative resting measurements of heart rate, respiratory rate, rectal temperature and body weight were obtained. Sedation was scored both inside and outside a kennel prior to drug administration and at 10, 20 and 30 min, post-drug administration. Dogs were assessed for behavioral responses (leg withdrawal, shivering, rigidity, orienting, panting, struggling, vocalization, wide-eyed facial expression, breath holding, salivating, hiding, biting, or requiring a muzzle) during three time periods: placing the dog on the table, clipping and prepping of forelimb, and intravenous catheterization. Postoperative recovery behaviors were scored. Expired halothane concentrations were recorded at 15, 30 and 45 min, postinduction. Significant differences occurred in the level of sedation at 30 min, between dogs receiving nalbuphine/xylazine (P = 0.005) or xylazine (P = 0.02) compared to dogs receiving acepromazine. There was a significant difference in behavioral scores between dogs receiving nalbuphine/xylazine compared to dogs receiving xylazine with respect to leg withdrawal (P = 0.02) and orienting (P = 0.014) during clipping/prepping. The combination of nalbuphine and xylazine is a useful premedicant, which provided greater sedation compared to acepromazine, and reduced some anxiety behaviors more than xylazine alone. Nalbuphine is an inexpensive opioid and is currently not a controlled substance in the US.
**P90 Complications of a Rabbit Surgical Model of Arteriosclerosis Using Periarterial Silastic Cuffs**

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Surgical placement of silastic cuffs around the carotid arteries in rabbits is an accepted model of arteriosclerotic disease. The silastic cuff induces a proliferative neointimal lesion resembling the biochemical and morphologic changes of early human atherosclerosis. At our institution, the model was used in a research protocol performed on Pasteurella-free NZW rabbits, and several serious complications occurred. Complications included inadvertent surgical trauma to vessels, acute respiratory distress that typically began 2-3 days after surgery, and sudden death. On necropsy of rabbits that died or were euthanized for respiratory disease, four rabbits were diagnosed with foreign body bronchopneumonia, and two of the four rabbits were diagnosed with vagal nerve degeneration. Aspiration pneumonia has been associated with esophageal dysfunction secondary to experimental vagotomy of the cervical portion of the nerve. The interval between surgery and respiratory disease and the presence of plant material in the lung was consistent with a proposed pathogenesis of denervation-induced dysphagia and subsequent acute aspiration pneumonia. The surgical procedure was carefully reviewed by the staff veterinarians, and in subsequent surgeries, careful attention was paid to dissection of the carotid artery from the fascial sheath that also encloses the vagus nerve. This modification of the surgical procedure has eliminated serious postoperative complications. This straightforward surgical model can consistently produce carotid arteriosclerotic lesions, and with careful attention to minimizing surgical trauma to the vagus nerve, serious postoperative complications can be avoided.

**P91 Development, Maintenance, and Use of Animal Morbidity and Mortality Databases: Valuable Tools for Health Surveillance**

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The goal of the attending veterinarian is to evaluate the health and well-being of all animals, and provide adequate care. This can be an overwhelming task when dealing with large populations. Therefore, morbidity and mortality databases were developed at our institution to facilitate evaluation and tracking of animal health reports and death notices. The husbandry and technical staff submit animal health reports and death notices to the clinical veterinarian. This information is entered into a database and the animal health report or death notice is assigned a tracking number by the database. In the case of the health report, this tracking number is written on the animal health report and on the red health cage card, identifying the animal as a clinical case. In addition when the animal is first evaluated by the clinical veterinarian or veterinary technician, a 0.5 in. round white dot is placed on the red health card, so whether or not an animal has been clinically assessed can be immediately identified. The animal health reports are placed in a binder with index tabs by room and rounds are made on a weekly basis, or more frequently if needed. Health reports are complete when the animal is euthanized or recovers. Investigators are initially notified with a report generated from the database when the information is first logged into the database. Weekly evaluations are e-mailed to investigators regarding the animal’s clinical progress. These databases are queried on a weekly basis to produce weekly morbidity and mortality reports for the rodent colonies. The report shows the number of clinical cases or deaths for each day of the week, the reporting staff member, the investigator, room location, reported problem, diagnosis, treatment, and case resolutions. These reports have been a valuable tool for health surveillance of our large rodent colonies.

**P92 Fibrosarcoma in a New Zealand White Rabbit**

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Rabbits are commonly used in biomedical research to generate polyclonal antibodies to a wide variety of compounds. The practice by some laboratories to maintain these indefinitely has provided our veterinary staff with the opportunity to diagnose more late-life disorders of rabbits than typically seen in a biomedical research facility. An adult female rabbit, approximately 5 years old, was used to generate antibodies against murine IL-10 for 4 years. The rabbit was reported to the veterinary staff for a mass on the right lateral thigh. The mass was firm, nonulcerated and attached tightly to the thigh musculature. The mass enlarged over the next three weeks, measuring approximately 6.5 x 5.5 x 1.5 cm, and a surgical biopsy was performed to obtain a histopathological diagnosis. Based on the presence of numerous spindle-shaped neoplastic cells interwoven between dense connective and adipose tissue, the mass was diagnosed as a fibrosarcoma. Because of the rabbit’s age and the complications associated with surgical removal and/or chemotherapy, it was decided to observe the animal closely until the condition required further medical intervention. Over the next six months, the only development was gradually decreasing weight and partial anorexia. The original mass had enlarged over that time, and a small, ulcerated mass was detected on the rabbit’s left shoulder (obscured by the pelage). The laboratory decided to euthanize the rabbit. At necropsy the rabbit had a large (7 x 7 x 2 cm) mass on the right lateral thigh and a small (2.5 x 2.5 x 1.5 cm), ulcerated mass on the left shoulder. Both of these masses were identified as fibrosarcoma, as they had the same histomorphological features as described for the biopsy. The lungs contained multifocal masses that were confirmed to be neoplastic metastases based on similar morphology and Trichome staining that validated the presence of collagen. Tension lipidosis noted on the surface of the liver was considered an incidental finding. This is the first reported case of a spontaneous fibrosarcoma in a rabbit.

**P93 First Isolation of CAR Bacillus from Rat Colonies in Argentina**

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The present study was carried out in 40 Sprague Dawley rats obtained from 8 commercial and experimental colonies. A routine microbiological monitoring (screening) of all the rats showed a positive result indicating the presence of cilia-associated respiratory (CAR) bacillus. To confirm the presence of the mentioned infectious agent, animals were immunosuppressed by applying cortisone (15 mg TD). After a period of...
6-8 days from inoculation, clinical symptoms of the disease were evident in all the rats. To further confirm the presence of cilia-associated respiratory (CAR) bacillus, symptomatic animals were euthanized. Homogenates were prepared by using trachea and lung. The homogenates were inoculated intranasal to eight rats free of CAR bacillus. All the inoculated rats shown symptoms of the disease after one week of the homogenate administration. The present data confirm the isolation of CAR bacillus in Argentinean rat colonies.

P94 Gas Bubble Disease and Secondary Red Leg Disease in a Colony of African Clawed Frogs (Xenopus laevis)

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An outbreak of red leg disease occurred in a colony of 200 African Clawed Frogs (Xenopus laevis) at the University of Wisconsin during January 2002. Several animals were submitted for necropsy. Two species of Aeromonas were cultured from the hemorrhagic lesions located on the hind legs and abdomen of the frogs, and small gas bubbles were noted in the webbing of the hind feet. No bacteria were associated with the gas bubbles. Water quality was evaluated and chlorine was found. The carbon filter was replaced and water was retested and was negative for free or bound chlorine. The cause of the red leg was believed to be due to a water quality failure. In February, many frogs were floating in their tanks with remarkable enlargement of the hind legs and an inability to stay submerged. The most distended animal was submitted for necropsy. Bubbles were present in the webbing of the feet and the pericardium was distended with gas. Gas bubble disease was diagnosed. This phenomenon has been described in Xenopus kept in water supplied by deep wells saturated with argon and nitrogen. Other sources of gas include supersaturation with oxygen, which is common in Wisconsin lakes in winter, or saturation with air when cavitation occurs due to leaks around pumps or small holes in water supply pipes within buildings. Airstones, commonly used in aquaria, were immediately added to each tank. The bubbling from these stones reduced the saturation of gases and protected animals from gaseous distention. The long-term solution will be the addition of a column aerator to the water filtration system or a change to RO water.

P95 Induced Agression in New Zealand White Rabbits from Feeding Reprocessed Commercial Diet

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One hundred and twenty male New Zealand White (NZW) rabbits weighing 2.5-3.0 kg were fed specialized test diet containing 1% cholesterol prepared from a pelleted commercial diet. Reprocessing the commercial diet was performed by a local feed processing laboratory. Reprocessing consisted of grinding the pelleted diet to powder, adding 1% cholesterol (w/w), diluting the mix to a slurry and pelleting the mixture. No vitamin-mineral mix was added to the slurry prior to the second pelleting process. Rabbits were fed this specialized diet for 6-8 weeks followed by a 6- to 8-week feeding period of the non-processed commercial diet. These alternating feeding regimes were repeated for 10-12 months. Within a few weeks of commencing the first 1% cholesterol diet feeding period, rabbits showed clinical signs of reduced feed consumption, diarrhea and aggressive behavior. These clinical signs subsided when the rabbits were placed on the non-reprocessed commercial diet. A review of the scientific literature suggested the clinical signs and particularly the incidence of aggression were due to hypomagnesemia. Feed analysis of the reprocessed diet showed magnesium levels were 50% of the recommended dietary allowance (RDA) for laboratory rabbits. Serum analysis of individual rabbits also revealed significantly lowered magnesium levels in rabbits showing clinical signs. Following feed analysis and serum chemistry data confirming the hypomagnesemia condition, the commercial diet manufacturer was contracted to produce the 1% cholesterol diet in 40-bag lots. This formulation had vitamins-minerals mix added to RDA levels for laboratory rabbits before pelleting. No problems with palatability or clinical signs of hypomagnesemia occurred during the remainder of the study. It was concluded that reconstituting the reprocessed diet to a slurry prior to the second pelleting process caused the leaching effect on the mineral content of the diet. This case study demonstrates potentially serious nutritional problems can be caused by reprocessing original diet.

P96 Necrotizing Laryngitis in Three Squirrel Monkeys

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Three young adult male squirrel monkeys (Saimiri sciureus) presented in respiratory distress over a period of 8 days. On physical examination, findings were normal except for severe hypothermia in monkey 1 and pronounced inspiratory wheezing heard on auscultation of all three monkeys. Each monkey was sedated for an oropharyngeal exam, radiographs, and blood collection. The monkeys had varying degrees of swelling and hyperemia of the larynx and epiglottis. Radiographs of the chest and neck were normal, but all monkeys had mature neutrophilias and elevated CPKs. The condition of monkey 1 was critical and it expired following intubation. Monkeys 2 and 3 were treated for possible anaphylaxis-induced laryngeal edema and bronchoconstriction but there was no improvement. Broad-spectrum antibiotics were administered for possible bacterial infection. The condition of monkeys 2 and 3 deteriorated and they were euthanized the next day. Gross necropsies of all three monkeys showed obstruction of the glottis by markedly thickened, pale tan, left laryngeal musculature and cartilages. Microscopic lesions in all monkeys were similar and consisted of extensive necrosis of the musculature of the left vocal fold with dense infiltration of neutrophils. Large numbers of neutrophils surrounded submucosal glands and focal areas of the mucosa were ulcerated. Lesions of the right side of the larynx were minimal. Tissue gram stains showed rare pleomorphic gram-negative bacteria. Cultures of the larynx grew Bordetella bronchiseptica, a gram-negative coccobacillus. B. bronchiseptica causes upper respiratory tract disease in diverse mammalian species and rare zoonotic infections. While it has not been definitively proven, B. bronchiseptica is considered the likely cause of these lesions because of its ability to produce multiple toxins that cause host cell necrosis. Investigations are underway to further characterize this strain and determine its role in pathogenesis.
P97 Necrotizing Pyogranulomatous Pneumonia in a p47-/- ApoE-/- Mouse Caused by Aspergillus terreus

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An adult female p47-/- ApoE-/- mouse was presented to the Division of Laboratory Animal Medicine at the University of North Carolina at Chapel Hill School of Medicine for diagnostic evaluation. The mouse was 1 of 28 in an investigator’s breeding colony, 6 of which died and were examined. Investigators noted more frequent deaths than are seen usually in this group of animals. Clinically, the mice were tachypneic, thin and hunched with dull, unkempt coats and had evidence of dehydration. At necropsy, the lungs failed to collapse upon opening the thoracic cavity, were discolored grey/white, and contained multifocal (diameter, 0.2 to 0.5 cm) raised white nodules replacing the parenchyma. There were multiple focally extensive adhesions of the lungs to the thoracic wall. Histologically, the lungs contained multifocal coalescing areas of severe pyogranulomatous infiltrate with multiple areas of necrosis. Periodic acid-Schiff staining revealed fungal hyphae within affected areas. Hyphae were 3-6 µm in diameter, frequently septate, and exhibited dichotomous branching. These features are morphologically consistent with Aspergillus spp. Fungal culture was identified as Aspergillus terreus Thom. Aspergillus terreus is commonly found in stored crops and soil. It has been reported to cause allergic or invasive bronchopulmonary aspergillosis as well as infections of eyes, nails, and skin. Experimental lesions have been reported in mice. In this case, a change in husbandry to autoclaved caging, bedding, food, and water along with eliminating affected individuals helped to eliminate the fungal problems within the colony. Given the current explosion in the use of increasingly complex genetically altered mice in research, great care must be taken to make accurate diagnoses in order to efficiently control problems as they arise. Diagnosis, husbandry, and genetics are discussed.

P98 Spontaneous Cardiomyopathy in a Cebus apella

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An adult, 2.8 kg, female, wild-caught Cebus apella with an intracranial chamber of two months duration was on a 9-week hiatus from a 19-month dystonia study when it died with no premonitory signs. Gross necropsy revealed thickened cardiac ventricular walls. The left ventricular free wall, right ventricular free wall, and interventricular septum measured 11 mm, 4 mm, and 10 mm, respectively. A sex- and weight-matched control had measurements of approximately half the thickness. The diameter of the left ventricular lumen was 7 mm, twice as large as the control. Additionally, necropsy showed fibroconnective tissue, 3-4 mm thick, between the cerebrum and the chamber lumen, which contained a brown, viscous fluid. However, the brain was grossly and histologically normal. The left adrenal gland, weighing 1.4 g, was twice the size of the right. Numerous Dipetalonema sp. were in the peritoneal cavity and heart. These nematodes are a common incidental finding in wild-caught Cebus. Histopathology demonstrated a mild adrenal hypertrophy, a mild myocytic hyperplasia of the papillary muscles in the left ventricle, and a septic thrombus in the right ventricle. The primary differential diagnosis was cardiomyopathy, possibly due to primary cardiomyopathy or secondary to a systemic hypertension. Cebus apella are used in Trypanosoma cruzi research as a model for the cardiomyopathy induced in the chronic stage of Chagas’ disease, which grossly resembles the lesions seen in this case report. However, no evidence of trypanosomiasis or its associated myocarditis was seen, despite its wild-caught status. Cebus with Trypanosoma cruzi often die due to a fatal arrhythmia induced by the cardiomypathy, a similar arrhythmia is the suspected cause of death in this case. To the author’s knowledge, this is the first report of a spontaneous cardiomyopathy in Cebus apella and should be considered a potential confounder of trypanosomiasis research.

P99 Urinary Tract Disease in Lewis Rats (Rattus norvegicus) Housed in Non-Sterile Conditions

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A high incidence of chronic renal disease and urolithiasis occurred in a group of Lewis rats in our facility. All rats were involved in an intracranial Lymphoctic Choriomeningitis Virus (LCMV) study. Over three years, 52 rats were necropsied; 26 of them had clinical signs ranging from urine scald to sudden death, and 26 were clinically normal. Median age at necropsy was 22 weeks (range 4-52 weeks). Struvite uroliths were found in 12 (23%) rats and chronic renal disease was found in 30 (58%). Possible causative factors included infection with LCMV, sex, diet, or housing. Sex did not affect the development of chronic renal disease or uroliths, all rats were fed the same commercially available rodent diet, and all were housed in non-sterile BSL 2+ conditions. Rats infected with LCMV had a higher relative risk for developing uroliths (RR = 15.9, P < .002) and clinical symptoms were significantly more likely (RR = 7.5, P < 0.001). However, LCMV infection did not correlate with chronic renal disease (RR = 1.2, P = 0.545) or cystitis (RR = 2.1, P = 0.244). Necropsies of other strains of rats housed in similar conditions and fed the same diet did not reveal urinary tract abnormalities, suggesting a potential strain predisposition. In addition, some litters were significantly more likely to develop chronic renal disease (P < 0.002) and cystitis (P < 0.009), indicating a possible genetic component. Struvite uroliths have been associated with bacterial infection, and bacterial isolates were cultured from vulva (7/7), urine (6/12) and kidney (3/5). Proteus mirabilis was the most common species recovered. In preceding years, rats in this study had been housed in sterile conditions and had not shown signs of urinary tract disease. This report suggests that housing Lewis rats in non-sterile conditions increases the risk of developing ascending urinary tract infections and concomitant renal disease, and that LCMV infection greatly increases the risk of subsequently developing urinary calculi.

P100 The Development of a Dental Hygiene Program in a Laboratory Canine Breeding Colony

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The importance of a strong dental hygiene program for humans has long been understood. It is now recognized that
m maintaining the good oral health of dogs is of utmost importance. It is imperative to control periodontal disease and to provide regular oral exams for several reasons: to maintain the teeth and gingiva in a healthy functional state, to minimize tooth and bone loss, to prevent oral discomfort, to detect oral masses or inflammation, and to guard against the spread of infection from the oral cavity throughout the body. Establishing a comprehensive dental program at our laboratory canine breeding facility was a complex task because of the large number of animals involved, with many at various stages of gestation and lactation. First, a dental staff dedicated to this program was selected and provided with in-depth training, including performing oral exams, identifying degrees of periodontal disease, and administering treatments and medications. Second, a computer-based program for monthly identification was designed to target broodstock most in need of dental attention. Each month a new list is generated and assessed to identify newly bred and pregnant bitches, newly weaned bitches, wet nurses, animals that have not had their teeth examined in a year, and any special needs animals. All dentals are performed under carefully monitored anesthesia using an ultrasonic cleaner. Surgical extractions that are necessary are performed by the staff veterinarian. Finally, each dental procedure is thoroughly documented. Missing teeth, exposed bone, oral masses, extractions and medications given during and after the dentistry, are recorded and become part of that particular animal’s permanent record. We have found that a comprehensive dental program in our laboratory canine breeding colony has lead to the better overall health of the individual animal and the colony, thereby producing and maintaining a better product.

**P101 Comparison of the Efficiency of Introducing Foreign DNA into Eggs of Inbred C57BL/6 and Hybrid BDF1 Mice**

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The production of transgenic mice by microinjecting foreign DNA into fertilized eggs is an indispensable research tool in modern life science. Between 1997 and 2000, we created more than 2,500 transgenic founder mice with over 350 types of foreign DNA. To generate these founders we used mainly fertilized eggs of inbred C57BL/6 and hybrid BDF1 (C57BL/6xDBA/2) mice. In the present study, we totaled the results of founder mice produced using fertilized eggs of these two strains, then compared the efficiency of introducing foreign DNA between the two. Transgenic founders were generated as follows. The vector portion was cleaved and purified linear DNA (5 ng/ul) and then genomic DNA extracted from the tails of weaned pups was microinjected into the male pronucleus of one-cell eggs. The state of the eggs was observed after 1-2 h, and eggs in good condition were transplanted into the oviduct of pseudopregnant ICR mice. These surrogate mothers gave natural birth, and then genomic DNA extracted from the tails of weaned pups was compared. The mean DNA introduction rate (%: transgenic founders/ transferred eggs) was 3.6% with BDF1, against 1.7%, or less than half, with C57BL/6. The mean birth rate (%: pups born/ transferred eggs) was 24.5% with BDF1, against 13.1%, also only about half, with C57BL/6. Thus, the low DNA introduction efficiency of C57BL/6 is thought to reflect the low birth rate in this strain. To improve the efficiency of introducing foreign DNA into C57BL/6 mouse eggs in the future, we plan to investigate pseudopregnant surrogate mother mouse strains.

**P102 Duck Anesthesia: A New Frontier for Laboratory Animal Technicians**

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Due to the complex nature of avian pulmonary anatomy and physiology, unique challenges may arise when anesthetizing ducks. For example, it is difficult to maintain ideal O₂ saturation throughout the entire surgery without providing assisted ventilation. Assisted ventilation is best accomplished manually at a rate of 4 to 6 breaths per minute. The volume administered is best judged by observing the breast of the duck.

Maintaining heart rates on any animal during surgery is unpredictable, and duck heart rates seem to fluctuate more readily than mammals. Appropriate anesthetic level, and adequate ventilation provide stabilization of the heart rate. When monitoring O₂ saturation as a measure of adequate ventilation and heart rate as a measure of depth of anesthesia, stable consistent readings are obtained when the pulse oximeter is placed on the tongue.

Ducks have a normal body temperature of 104°F to 107°F, and may experience a high rate of heat loss during anesthesia. To maintain body temperature and prevent heat loss we eliminated alcohol during preop scrubbing, plucked minimal amounts of feathers, and used circulating hot water blankets during surgeries. Special care should be emphasized on placement of a rectal thermometer because accidental entry into the oviduct of females can cause infection in the reproductive tract.

Ducks are used as toxicological and pharmacological models. In our facility they are used in hepatitis research and require liver biopsies at various stages. We have successfully improved survival rates by becoming familiar with the anatomical traits of the duck and avian anesthesia concepts. Adequate knowledge of anatomy and physiological monitoring during anesthesia reduces the loss of animals and allows for successful use of this species in our research endeavors.

**P103 Enhancing Postsurgical Recovery of Pair-Housed Nonhuman Primates (M. fascicularis)**

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In many facilities, postsurgical protocol in the nonhuman primate requires individual housing for a period of 2-10 days, depending on the procedure. In pair and group-housed animals, this becomes an even more stressful event upon recovery from the surgical procedure. The additional burden of being removed from its pair or group for a short period of time can have adverse effects (e.g., weight loss, gastrointestinal issues, increased stereotypic behavior). The longer the animal is separated from its group, the greater the potential to observe aggressive behavior when returned to its original social setting. Our goal was to allow same-day return of the postoperative candidate to its paired environment. Fifteen female macaques (M. fascicularis) ranging between 5-6 years of age were instrumented with vascular access ports between June and December 2001. Postoperatively, the animals were placed into cloth jackets and once totally recovered (righting reflex established, mobile without signs of ataxia), returned to their social group setting.

Change in hierarchy status, self-traumatic events, weight loss or diarrhea did not occur in any of these animals, and the inci-
sion sites healed unremarkably. The animals ate and drank normally, and received their postoperative treatments without problem (readily accepted oral medication).

We conclude that this practice of quick return to group status postoperatively can be successfully employed, and is a “best practice” when working with these laboratory animals.

**P104 “Burnout and Breeding” Program for MHV-Infected Transgenic Mice**

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Because viral, parasitic, or bacterial infections of any kind present a potential health hazard for laboratory animals, research animal facilities often take extreme precautions to prevent introduction of such infections into animal colonies. Clinically significant infections often spread rapidly through colonies and may either compromise animal health, resulting in death, or may adversely affect the animals, rendering them useless for research purposes. Even subclinical infections can significantly affect research and invalidate years of data. Outbreaks of Mouse Hepatitis Virus (MHV) are especially devastating in research mouse colonies because this infection spreads so rapidly and is difficult to contain. In most cases, infected animals can no longer be used for research purposes. A single outbreak can cost an institution thousands of dollars in loss and replacement of animals, follow-up testing and cleanup, and can delay research progress by months if not years. Many transgenic strains may not be easily obtained or may be replaced only by rederivation. If successful, a “burnout and breeding” program could provide an alternative procedure for saving certain strains of transgenic mice affected by an MHV outbreak. Although infected mice are themselves useless for most research purposes, once they are no longer shedding active virus (“burnout”), if bred, they can produce uninfected offspring that can be used for research applications. For the program, one facility was designated as the “burnout” area. All infected adult transgenic mice were housed in one room. After the appropriate period of time following the outbreak, breeding pairs of various transgenic mouse strains (20 different types) were set up. Pups resulting from successful matings were weaned at 4 weeks and separated by gender. Pups were monitored for MHV infection by serologic testing (IFA and ELISA). Serum samples were obtained by survival bleeding one pup from each litter using the retro-orbital technique. Samples were taken at specific ages: 4 weeks, 8 weeks, and 2-week intervals thereafter for a total of 3 consecutive negative tests. First-generation offspring, which remained seronegative throughout the testing period, were transferred to the designated “step-down” room and were tested at 2-week intervals. Subsequently, breeding pairs of first-generation pups were set up and second-generation offspring resulting from successful matings were monitored for MHV infection by the method previously described for first-generation pups. Those first- and second-generation pups remaining seronegative were transferred to a restricted-access corridor in another clean facility. Once in this room, to eliminate the risk of losing experimental animals, all animals were monitored routinely by a modified sentinel program: one sentinel cage (2 animals) with microisolator top was set up for each investigator. Dirty bedding from all cages belonging to one investigator was sampled into the designated sentinel cage for that investigator. The “burnout and breeding” program had favorable results: of the 49 first-generation litters, 33 were seronegative for MHV; 8 were seropositive for MHV; and 8 litters died or were euthanized before testing was completed. The data indicates that MHV infection can be “burned out” in some transgenic mouse strains. Overall results suggest that a “burnout and breeding” program can be used successfully as an alternative method to rederivation for saving some MHV-infected strains of transgenic mice.

**P105 Effectiveness of Passive Gas-Scavenging Canisters Attached to Isoflurane Anesthesia Systems under Standard Use Conditions in a Laboratory Animal Facility**

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Chronic exposure to trace levels of waste anesthetic gases has been linked to higher incidences of neurologic and reproductive dysfunction, hepatic and renal toxicity, and neoplasia in health care professionals. We have shown that low-level isoflurane emissions are likely in conventional laboratory animal treatment rooms during the use of standard anesthesia delivery systems equipped with activated charcoal canisters for passive gas scavenging. In the present study, we surveyed the effectiveness of canisters (attached to well-maintained precision isoflurane vaporizers) in current use throughout our AAALAC-accredited laboratory animal facility. Canisters (Omnicon f/air; A. M. Bickford, Wales Center, NY) had been weighed prior to use and then attached to dual-loop systems (facemask and induction box circuits) for from 1 week to 6 months of service. (Canisters had been placed in or near chemical fume hoods to offer additional emission control.) Isoflurane emissions were measured using a portable infrared spectrophotometer by attaching each canister to the facemask circuit, occluding the facemask and closing the stopcock to the induction circuit, and running the system at uniform isoflurane concentration (2%) and oxygen flow rate (1 L/min). Samples were taken in animal procedure rooms (size range, 45-80 m2) in which the air turnover rate ranged between 20 to 30 non-recirculating changes per hour. Nine of 60 canisters (15%) in current use were found to have exceeded the manufacturer’s recommended use-life (defined as a weight increase of 50 g). Of these nine, seven canisters did not scrub isoflurane at all (indicated by emissions greatly exceeding 100 ppm). Isoflurane was not detected in the operator’s breathing zone throughout our AAALAC-accredited laboratory animal facility.

**P106 Acetaminophen-Induced Hepatocellular Necrosis: A Comparison in Male Sprague Dawley, Wistar and Lewis Rats**

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Sprague Dawley, Lewis, and Wistar (Charles River, Raleigh, NC) rats, which are commonly used in biomedical and pharmaceutical research, were evaluated for their tolerability of two therapeutic doses (100 mg/kg or 300 mg/kg, PO) of acetaminophen. Forty-five age-matched male rats (5 rats/dose/stock or strain) were orally dosed q12h for one day. Control rats received saline at an equivalent oral dose volume, frequency and duration. Twelve hours after the second dose, blood for chemical analysis evaluation was collected by cardiac puncture following CO2 euthanasia. The left lateral lobe of the liver was taken from each animal for histological evaluation. Clinical chemistries results were within the normal range compared to vehicle controls for all rats. Histological findings showed a moderate, yet statistically significant, hepatocyte necro-
sis/degeneration and centrilobular inflammation in the Lewis rats at the 300 mg/kg dose level. In Wistar rats, there was a marginal histological finding at 300 mg/kg. No abnormal findings were seen histologically in the Sprague Dawley rat livers at either dose level. This suggests that the recommended therapeutic dose and frequency of acetaminophen in rats (100-300 mg/kg PO, q4h) has the potential to induce histological hepatic necrosis in certain stocks or strains of rats at the higher dose and/or with frequent dosing. Hepatic necrosis may impact subsequent studies involving liver; i.e., pharmacokinetic parameters.

P108 Pair-Housing Rabbits in Standard Laboratory Cages: The Relative Importance of Social Enrichment

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Although The Guide for the Care and Use of Laboratory Animals clearly indicates that washing and disinfecting of cages by hand is acceptable, protocols for accomplishing sanitation by this method vary considerably, as does their effectiveness. This study was designed to evaluate the effectiveness of a hand-washing protocol for cages by monitoring adenosine triphosphate (ATP) concentrations on surfaces. The concentration of ATP was correlated with plate counts taken from the same surfaces and was found to be consistent. Two hundred dirty rat cages were used in the protocol for hand-washing of cages. Forty-four cages were selected from the total cages washed based upon their position in the cage-washing process, and the values of ATP before and after washing were assessed using an ATP meter and swab collection system. An average 97.5% reduction in ATP levels was achieved, which correlated well with RODAC plate values. While many methods of cage washing and disinfection can be used to achieve the goals of adequate sanitation, it is necessary to calibrate/validate such processes to ensure the desired outcome.

P109 The Effects of Cage Population on the Weight Gain and Food Consumption of Male Sprague Dawley Rats Housed in Wire Cages

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The objective of the current study was to evaluate the effect of cage population on food intake and weight gain of male Sprague Dawley rats housed on wire cages. All the rats were housed in the same 16 × 10 × 7 (W × D × H) wire cages, and the area of the floor space was 160 in². The hypothesis was that cage population would have an effect on weight gain as well as food intake because of stress due to the cage population. Rats weighing 200-225 g were received from Taconic Farms. The rats were acclimated for 5-7 days prior to study in a room with temperatures ranging from 64-79°F and humidity at 30-70%. The rats were provided with water in bottles ad libitum. All food was measured per cage.

Rats were arranged randomly in four groups: group 1, one rat per cage; group 2, two rats per cage; group 3, three rats per cage; and group 4, five rats per cage. The experiment was a 26-day study and consisted of: weighing animals at approximately the same time every day, weighing the amount of food put into troughs and weighing the amount of food left over from the day before. All food and rats were weighed on a top load scale.

The results of the study indicated that the average food consumption per rat did not differ significantly when compared by ANOVA analysis at P < 0.05. The range of food consumption per rat was from 25-31 g per day. When the weight gain of the 4 groups was analyzed using ANOVA analysis at a significant value of P < 0.05, the absolute change in weight between groups was not significantly different. The average weight gain per rat ranged from 116-131 g over the course of the study. In conclusion, the data indicated that the number of rats per cage did not have an effect on the food consumption or weight gain in Sprague Dawley rats.

P110 The Effect of an Environmental Enrichment Device on Individually Caged Rabbits in a Toxicology Facility

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The primary enclosure of a laboratory animal’s environment should encourage species-typical behavior and enhancement of the animal’s well-being, as indicated by the Guide. Enrichment devices have been documented to decrease the incidence of stereotypical behaviors and increase overall activity of rabbits. We performed a study to evaluate the effect of an environmental enrichment device, stainless steel rabbit rattles on spring clip, in individually housed rabbits in our toxicology facility. Forty-eight NZW rabbits were used. Parameters evaluated included: device manipulations, food consumption, body weights, and the evaluation of hematologic parameters for the stress triad. No significant differences were found between study and control.
rabbits when body weights, food consumption and hematologic parameters were analyzed. Our study supports previous findings that interaction with enrichment devices decreases over time, thus indicating the need for frequent rotation of different enrichment devices. Additionally, no adverse effects of the analyzed parameters were found, indicating that stainless steel rabbit rattles on spring clip are suitable devices for toxicology studies where introduction of new variables is often unacceptable.

**P111 Cost Comparison of Fenbendazole and Ivermectin for Pinworm Eradication**

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At our institution we give the investigators the option of treating *Syphacia* and *Aspiculuris* pinworm outbreak with either fenbendazole rodent chow or ivermectin-mediated water. The cost of medication is calculated on the average rodent population per box: 5 per box (mice) and 2 per box (rats). Treatment costs with fenbendazole are calculated on a week of medicated chow and then a week of non-medicated chow for a total of 5 weeks. The animals will therefore have the chow for a total of 21 days as their sole food source. Both regular and breeding fenbendazole rodent chow are available. The cost of the regular fenbendazole rodent chow per box is $4.86 (mice) and $5.54 (rats). This makes treating an average mouse room $1944.00 (400 boxes) and an average rat room $775.00 (140 boxes). Using fenbendazole breeding chow raises the price to $11.03 a box (mice) and $12.60 a box (rats). This makes treating an average room $4412.08 (mice) and $1764.00 (rats). Treatment costs with ivermectin are calculated by three 3-day periods where the medicated water is the sole water source, over a 3- to 5-week period. The cost of ivermectin per box is $1.20 (mice) and $2.40 (rats). This makes an average mouse room $480.00 and an average rat room $336.00. These price comparisons demonstrate that ivermectin is more cost-efficient; however, investigators may find fenbendazole more advantageous due to the fact that it is non-toxic and involves less labor. Armed with this information, the investigators choose what is best for their particular rodent colony.

**P112 A Comparison of Microbiological Culture to Adenosine Tri-phosphate Hygiene Measurements in Evaluating the Effectiveness of Facility Sanitation Procedures**

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Effective animal facility sanitation programs are essential to safeguard animal and human health and to minimize the risk of adversely effecting research studies. We conduct periodic microbiological monitoring of animal rooms, cages, and equipment after chemical sanitization, rack or tunnel washing. Bacterial contamination is evaluated through culture media and RODAC plates. While RODAC plating is a very sensitive and effective tool, the major disadvantage is a 24-48 h delay waiting for bacterial growth. Adenosine Tri-phosphate (ATP) is a chemical associated with all biological materials. Surface measurement of ATP is regarded as a highly sensitive indicator of sanitization effectiveness in the food service industry. Portable analyzers, called luminometers, can provide an indication of the levels of ATP present on a surface in under a minute. Over the past year we have compared over 750 RODAC plates and ATP hygiene measurements at our facilities. We set the following limits to qualify as an acceptable level of sanitation: RODAC ≤ 5 colony growths per plate. ATP levels < 1000 Relative Light Units (RLU) on walls and floors, < 500 RLU on plastic and 0 RLU on glass or stainless steel. In every instance when we recorded a FAIL on RODAC tests, we also recorded FAIL on ATP measurements. Every recorded PASS on ATP measurements also achieved a PASS on RODAC tests. We saw a 99% PASS rate with RODAC tests on floors only to have 22% of floors PASS the ATP measurements. Recleaning, in some cases 2-3 times, would eventually return an ATP hygiene PASS result. ATP measurements appear to be a more sensitive indicator of sanitization and provide instant feedback. A room or piece of equipment that does not pass can be recleaned immediately, eliminating the risk of reuse in a less than sanitized state.

**P113 A Novel Environmental Enrichment Program at the Oregon National Primate Research Center**

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In the wild, nonhuman primates (NHPs) spend much of their day foraging for food. This time is often greatly reduced in a laboratory setting. In an effort to increase the foraging opportunities available to caged monkeys, the Oregon National Primate Research Center initiated a novel enhanced Environmental Enrichment (EE) program. Devices that promote foraging and manipulation, such as “Feeders,” “crumble disk holders” and “challenger balls” are hung on cages for one-week time periods on a rotating basis (i.e., one week with a device, followed by a week without a device), giving animals access to new foraging manipulanda every other week. The devices are filled with items such as trail mix and vegetables. When the devices are not present, the monkeys are provided with other forms of enrichment. We started this program in approximately half of the monkeys at ONPRC. In a retrospective study, we tested the hypothesis that animals diagnosed with “over-grooming” (an atypical behavior in which monkeys pull out their own hair) that had access to the EE program (n = 10) would show improvement compared to those that did not have access to the program (n = 12). Behavior technicians assessed these monkeys weekly using a standardized rating scale (0-5; 0 = no hair loss, 5 = more than 75% of hair missing). We averaged these scores before and after the initiation of the program. A 2-factor repeated measures ANOVA revealed an interaction between over-grooming score and treatment (F(1,20) = 4.10, P = 0.057). Over-grooming monkeys that had access to the EE program showed improvement over time, while the others remained the same. These preliminary data suggest that our Environmental Enrichment program is having a positive effect on monkeys that engage in over-grooming behavior. More work is needed to determine how this program affects monkeys without, or with other, atypical behavior patterns.

**P114 Elimination of Pinworm Eggs from Caging Equipment with Vaporized Hydrogen Peroxide**

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Infestation by oxyurid nematodes is often detected in laboratory rodent colonies. The environmentally resistant eggs aerosolize easily, persist for longer periods of time and are hard to eliminate by common disinfectants. This study was performed to find out whether fumigation with vaporized hydrogen peroxide can successfully inactivate pinworm eggs on contaminated
caging equipment. These experiments were carried out in triplicate with three groups of five endoparasite-free male SCID mice each. They were exposed to soiled bedding from *Syphacia muris* positive rats, housed in ventilated racks and serviced under sterile conditions. Prior to animal transfer these dirty cages were treated in three different ways: 1. Test group: fumigation with hydrogen peroxide for 15 min. at 30°C (total amount of 8.27 g per cubic meter); 2. Negative control group: autoclaving for 20 min. at 121°C; 3. Positive control group: no treatment. After 2 weeks of exposure all animals were returned to clean cages. Four weeks later perianal cellophane tape tests were taken, and after another two weeks all animals were sacrificed for direct visualization of cecal contents. All 15 animals housed in the untreated dirty cages were positive for *Syphacia* eggs on perianal tape tests and were heavily infested with adults and juvenile pinworms on visualization of cecal contents, whereas all 30 mice kept in autoclaved and hydrogen peroxide treated cages stayed negative during the study period. None of the mice showed obvious health problems. These results clearly demonstrate that fumigation with hydrogen peroxide completely prevents pinworm eggs from infesting susceptible hosts. This method can be recommended for effective elimination of pinworm eggs from contaminated caging equipment and as one step further from animal rooms.

**P115 Employee Suggestion Program**

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Our institution solicits input from employees through its “Employee Suggestion Program.” The purpose of the program is for employees to become frequent and successful participants in the workplace.

In our organization, we have team meetings and agenda meetings on a routine basis. In the team meetings, the animal technicians meet with their team supervisors and share their views and insight. There are certain useful suggestions from the staff the supervisor thinks could be an appropriate topic for discussion for the next agenda meeting. There are two primary arenas in which employee suggestions are presented: at the weekly team meeting or the bi-weekly agenda meeting. Team meetings are informal, comprised of animal caretakers and their immediate supervisors. In these meetings ideas are brought forth, discussed and formalized to be taken to the next step. The agenda meeting consists of representatives of administration, faculty, clinicians, support staff, animal technicians and attendants. Employee suggestions raised here invite discussion and thought. While not all suggestions are implemented, employees receive valuable feedback, which may help shape future ideas and suggestions. Some of the employee suggestions that have been implemented are a flexible shift time, 3-day workweeks, recycling of empty bottles, use of rechargeable flashlights, and preassembled cage supply orders. Additionally, management has incorporated such employee ideas as temporary promotion program, stand-in or “floaters” technicians who cover absences, and a peer-mentoring program to orient and establish new employee in their new work environment. The Employee Suggestion Program strives to eliminate every form of waste. It provides an opportunity for employees to develop and demonstrate their initiative and ingenuity. It lets employees share in the benefits that result from the adoption of their ideas. The most important outcome is teamwork and joint action through proper communication.

**P116 Laboratory Canine Good Citizens: A Socialization and Training Program for Dogs at the NIH**

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A socialization and obedience training program is beneficial for both dogs and animal care staff in any laboratory situation. Obedient laboratory canines are easily handled by caretakers and can be trained to cooperate in a variety of procedures including walks to the treatment room, nail trims, and vaccinations. During the socialization and training process, the dogs gain exercise and enrichment from their human trainers and interact with other dogs in a supervised environment. A socialization and positive reinforcement training program was begun at the National Institutes of Health Animal Center, Veterinary Resources Program, Canine Unit. The program represents a collaborative effort between the behavior staff and animal care staff to provide for the well-being of the laboratory canines. Dogs currently in the program range in age from 10 weeks to 2 years. Trainers use food, praise, and toys as rewards for various behaviors. Dogs learn behaviors such as sit, come, down, stay, and walk on a loose lead through various games based on positive reinforcement. Jumping up, biting, and mouthing are addressed and minimized, mostly by ignoring the inappropriate behavior. Members of the animal care staff, including caretakers, veterinary technicians, facility managers, and veterinarians, are responsible for 2-4 dogs. All staff members work with their dogs individually for short periods (15 min. or less) at any time during the workday. Group obedience classes are held once each week and are taught by the behavior staff. Both the canines and humans have benefited from participation in this program. Dogs are less distressed and cooperate during routine veterinary and husbandry procedures; these procedures in turn are more efficient for the animal care staff.

**P117 Novel Design to Improve Ergonomics and Reduce Facility Costs**

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Common tasks performed in animal facilities should be evaluated for their ergonomic impact and for possible modification to minimize costs. We evaluated the use of centrally supplied CO2 in a mouse facility procedure room. CO2 was supplied from a standard laboratory gas petcock mounted on the wall at the back of the procedure room countertop. The distance from the front of the countertop to the valve made it difficult to reach and users would not consistently turn off the CO2 after euthanizing mice. Our first solution was to install a push-actuated CO2 valve requiring users to hold the valve open for CO2 flow. Users would circumvent this by tying open the valve with a latex glove then failing to later remove the glove so that CO2 would be wasted. We then devised a remote means of operating the CO2 valve that would not be easy to tamper with, be easy to use and sanitize, and be ergonomically advantageous for all users. A foot pedal was remotely connected to the wall-mounted CO2 valve via a flexible cable allowing for a range of placement of the pedal for ease of use and ease of room sanitation. Design parameters included the operating range of the CO2 valve plunger from full off to full on and attachment of the mechanical linkage from a foot pedal to the existing valve hardware with sufficient travel to cover the full operating range of the valve plunger. The foot pedal system was assembled from parts available from a hardware store and from custom parts manufactured.
from aluminum. This hinged pedal manipulates a cam that displaces a cable connected to the mechanical linkage of the wall-mounted CO₂ valve which, when displaced, controls the flow of CO₂ and automatically shuts off when the foot pedal is released. This novel device has significantly decreased the use of CO₂ in the procedure room and elicited positive comments from users as to ease of use.

P118 Oversight of Department of Defense-Funded Animal Research in the Former Soviet Union

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Since the enactment of the Soviet Nuclear Threat Reduction Act in 1991 (renamed the Cooperative Threat Reduction [CTR] program in 1993) the Department of Defense (DOD) has been an integral partner in assisting the countries of the former Soviet Union destroy nuclear, chemical and biological weapons of mass destruction, associated infrastructure, and establish verifiable safeguards against the proliferation of those weapons. One component of this program is establishment of collaborative research programs with scientists formerly engaged in biological weapons research. Prior to conducting collaborative animal research at the institutes, DOD laboratory animal veterinarians were called upon to evaluate the current status of the animal care and use facilities and programs. The responsible DOD directive states that activities performed or sponsored in foreign countries shall be conducted in accordance with applicable U.S. statutory requirements, and regulations and standards of the host country. If differences exist between U.S. and host country regulations or standards, the more stringent standard applies. As a result of initial visits made in 1998 and 1999 to a number of Russian research institutes, the Director of Defense Research and Engineering (DDR&E) supported and signed the recommendation not to fund any animal research in the former Soviet Union until comparable animal care and use standards were implemented. Due to a lack of current research animal care and use guidelines or legislation within the former Soviet Union, both general recommendations and specific guidance were provided. These recommendations included the formation of an animal care and use committee (ACUC); written animal research proposals in a standardized format; animal care and use training for investigators, technicians, and members of the ACUC; the use of specific pathogen free animals; appropriate caging and accessories for the species and project; and renovations to the areas used for CTR projects. Some of the progress and pitfalls that occurred over the last four years are discussed.

P119 Sheltered Housing at the Oregon National Primate Research Center: A Novel Approach for Enhancing Nonhuman Primate Social Group Environments

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There has been an increasing awareness of the importance of social housing for nonhuman primates (NHP) in recent years. Group housing used for breeding purposes typically consists of either small indoor groups made up of several adult females and one male, or large outdoor corrals consisting of numerous adult females and males. The Oregon National Primate Research Center (ONPRC) has designed and built novel 1,300 ft² compartmentalized sheltered housing units to house our specific-pathogen-free (SPF) rhesus macaque populations. These new units offer monkeys more social opportunities than monkeys in paired caging, are more accessible than larger corrals and allow for closer monitoring of group dynamics. The units are outdoors, but are environmentally controlled, with roofs, overhead heating and heated floors. There are play structures on the two end sections, and the middle section can be enclosed for animal processing. To date, we have organized 9 groups of NHPs (average = 33 ± 6) into sheltered housing units. While these groups have relatively high density, we have seen little aggression associated with these group formations. Nineteen animals (6.4%) have been removed for injuries. We believe that one reason for this low rate of aggression is the presence of three distinct sections, which allow animals visual barriers from aggressors. The monkeys appear to display normative social behavior, although we are currently assessing this in greater detail. Further, we are training the monkeys to voluntarily cooperate with husbandry and experimental procedures, allowing them to stay in social groups even while assigned to scientific projects. Not only will these units improve the well-being of our monkeys, but they will also allow for an expansion of our NHP colony size due to increased reproduction success.

P120 Team Interviewing Method for Hiring Animal Care Technicians

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Hiring and keeping well-qualified entry-level animal care technicians is one of the challenges to maintaining a quality animal care program. Today’s job market provides an abundance of candidates from which to choose, but resumes often do not contain sufficient detail. Education and employment dates can be verified, but a mechanism is needed to determine if a candidate is honest, is looking only for a paycheck, or is an animal activist hoping to get a foot in the door. To better assess the suitability of candidates, we’ve developed a behavior-based, team interviewing process for selecting animal care technicians. The process evaluates a candidate’s past and present behaviors, and probes with questions that reveal his/her reactions to real-life situations. There are many opportunities to gather clues about a candidate. Even before the interview, a candidate reveals much in how he/she interacts on the telephone, is able to follow directions to get to the interview, meets (or doesn’t meet) at the specified rendezvous location on time, and how he/she dresses for the interview. On arriving at the interview the candidate is given a job description to read. Both interviewers introduce themselves and discuss how the interview will unfold. The team works from a set of questions it has developed to reveal fundamental behaviors: how the candidate interacts with customers and co-workers, his/her approach to conflict resolution, how he/she copes in the workplace, and what we might expect if that person joins our team. Each interviewer experiences the interview a little differently. With one team member leading with questions and the other recording answers, listening and observing are effectively doubled. While the lead interviewer focuses on the candidate’s answers, the second interviewer can focus on subtle but revealing qualities: if the candidate wears leather shoes, if the candidate holds eye contact or looks away, if he/she answers directly or is evasive and noncommittal. If the team feels comfortable that
the candidate is not an animal activist, they go on a brief tour of the facility. Observations are noted on how the candidate react to the sight and smell of hundreds of rodents, if he/she extends a hand to pet a dog or withdraws from the curious, friendly muzzle. Also noted are any questions the candidate asks while on the tour. After the tour, the interview resumes with more behavior-based questions and a chance for the candidate to follow up with his/her own questions. When the interview is over, the team then meets to discuss its impressions. Considered are the candidate’s strengths and weaknesses, his/her commitment to animals, and if he/she will enhance the husbandry team. Together with the impressions from the interview itself, the team decides if it wants to proceed with a check of professional references. While no hiring method is foolproof, our team feels it has been successful in hiring individuals who are committed to the animals in their care. The husbandry management team has responded favorably to the quality of employees gathered with this interview method.

P121 The Use of Marathon Oasis Rolled Rubber Flooring in a Laboratory Animal Facility Setting

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Our department conducted chemical and physical tests to see if the new flooring could withstand use in an animal facility. Marathon Oasis Rolled Rubber Flooring is molded from a combination of synthetic rubber, pigments, extenders, processing oils and fibers, and is packaged in rolls sized 1.5 m × 15.2 m × 2.0 mm. The flooring has a paper texture surface and conforms to various fire and electrical specifications. Installation is a three step process, involving adhesive being put down on various types of existing flooring, flooring being placed down, and after drying, seams are heat-welded and then sealed. Physical tests of the flooring included scratching and dragging heavy primate cages, rabbit caging, and stainless steel shelving along the floor. Chemical tests involved pieces of flooring being submerged in five different glass beakers containing common cleaning chemicals used in laboratory animal facilities, as well as urine and fecal material, for 24 h. The durability of this flooring in the short term has proved to withstand all physical and chemical tests conducted. However, long-term durability of this flooring has yet to be seen. Overall, the Marathon Oasis Rolled Rubber Flooring has proved to be beneficial to our animal facility attributable to its case of installation, maintenance, and durability.

P122 Using a Toy Rotation Scheme to Enhance Canine Enrichment

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Our challenge was to enhance an existing canine enrichment program for hounds singly housed 4-6 per room in 40 ft² runs. Historically, our program has consisted of rubber mats in each run, daily human interaction, and a daily exercise routine that allows for conspecific social interaction. Our goal was to improve the program by providing enrichment for those times without interaction. Our approach was to design a toy rotation scheme that would provide each dog with a different toy on a weekly basis. These toys needed to be durable enough to withstand weekly cage washing and prolonged chewing while measuring greater than 2.5 in. in height or diameter to prevent them from clogging our drainage system. Nine toys were chosen for evaluation: Best Ball, Buster Cube, Dental Ball, Dumbbell, Goodie Ball, Goodie Ship, Havaball, Hercules, and Kong. Each toy was evaluated by recording observations (2-3 times per day) on toy evaluation forms using evidence of sustained chewing, significant toy movement and dramatically increased play behavior as criteria. At the end of a 2-month evaluation period, only 4 out of the 9 toys (Dental Ball, Dumbbell, Havaball, and Kong) were completely successful at meeting our standards of durability and dimension while also providing lasting appeal to our canine population. A 4-week toy rotation scheme was then implemented and forms were designed for recording room number, weekly rotation day, month and year, identification of canine occupants by run, which toys had been placed for the week, and who had placed the toys. Our initial evaluation and continued observations suggest that the utilization of a toy rotation scheme has successfully improved our canine enrichment program by providing considerable distraction throughout the day. We believe that our toy rotation scheme would be a meaningful adjunct to any canine enrichment program.

P123 Waste Bedding Disposal System

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In all laboratory animal unit, the people who work there and all lab animals are in constant relationship with possible aerosolized allergens and other contaminants in normal macro environment conditions. In our laboratory animal facility, we design a system that minimize the risk of any allergen disease in technicians and veterinarians during the waste disposal soiled bedding moment, to protect them to allergies. This system have a capacity of 20 kg per minute of waste bedding All waste fall down into a jumbo plastic bag into the metal container. The total soiled bedding to fill at 75% of 55 gallons metal container is about 35 kg in 3 min. The exhaust air is filtered with two type of filters: one bag filter with two filter and one membrane filter with two filter. To know when the soiled bedding reach the top designed capacity, there is a vertical laser light to interrupt the electric energy when it reach 75%. In the filter banks there are two differential pressure sensors to evaluate the status of each type of filters.

P124 Wet/Dry Vacuum Aerosol Defeats Sanitation

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Frequently the surface areas (floors, walls, ceilings) of animal holding rooms are sanitized using chemical compounds that are applied in a variety of methods (spray, foam, mop, fog) to disinfect or sanitize. Often the remaining residual sanitizing product is collected using a wet/dry type of vacuum, particularly for areas without floor drains. These types of vacuums can aerosolize the compounds along with any other materials that may have been present in the tank from other uses during the vacuuming operation. The aerosol may contain contaminants from other areas prior to sanitizing. We tested the efficacy of wet/dry vacuums following sanitation by placing culture plates at several locations behind and around the exhaust blower port while collecting the residual sanitation product. Culture results from these plates revealed an increase in bioload when compared to the postsanitation cultures of surface areas. In addition, we found organisms that were not present initially (presanitation control
catalysts) that were introduced through the use of the vacuum. These results indicate that the aerosolization of the sanitizing compound allowed the introduction of undesirable organisms onto the previously sanitized surfaces. Using this type of vacuum can be counterproductive to sanitation, reintroducing organisms onto sanitized surfaces and in some cases causing the introduction of new and potentially infectious agents.

P125 A Simple System for Whole-Body Hyperthermia Treatment of Disease-Free Mice Using Conventional Facilities and Equipment

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We have examined the effects of heat stress during fetal development of mice with various Tpr53 status as part of a larger study on radiation and fetal effects. Large numbers of disease-free, timed-pregnant mice were transported from clean animal housing rooms to conventional research labs for whole-body heat treatment and returned without compromising their disease-free status. The system consisted of strict adherence to standard operating procedures (SOPs), an incubator, breathing quality air, rectal temperature probe and lubricant, sterile saline injections, a biological safety cabinet for unloading mice, filtered plastic housing/transport cages, two transport carts, sterile drapes, sterile bench liners, sterile lab coats and gloves, and 70% ethanol and quaternary ammonium disinfectants. This system allowed the researcher the flexibility to move animals out of the clean environment, treat them with heat outside the facility and return them to the animal facility in a disease-free state. Food and water were withheld during the heat treatment. Mice were injected intraperitoneally with 1 ml of sterile physiological saline to maintain hydration. The heating apparatus was a large incubator with a custom-made plastic glove box door to allow for manipulations inside the chamber. All surfaces were disinfected prior to treatment and sterile 0.2 µm-filtered, breathing quality air was delivered into the chamber to keep the interior positively pressurized and protect the animals from disease. The glove box door was temporarily removed for placement of the cages, and then repositioned. The filter tops were removed to allow for precise control of the animals' environment. Wire bar lids remained on the cages. The temperature was slowly increased over 30 min. to acclimatize the animals. Rectal temperatures were taken on random animals; once the average core body temperature reached 40.0 ± 0.5°C, the 60-min. heat treatment began. Following treatment, the animals were offered water and food and the temperature was slowly returned to 21°C. Clean filter tops were placed on the cages and they were returned to the normal housing. Hundreds of mice have been treated without introducing disease as confirmed using various health-monitoring tests. The equipment was readily available, easy to clean, reusable and appropriate for treatment of small lab animals while still maintaining disease protection of the animals.

P126 An Inexpensive Short-Term Housing System for Amphibians

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Syracuse University Principle Investigators use amphibians for short periods of time during the fall and spring semesters. The animals are housed for a period of up to a month. The standard housing for the amphibians were large rat boxes. The boxes would be partially filled with water and placed so that one end of the box was elevated allowing the animals access to a dry area. The water for each box had to be changed daily and the boxes at least three times a week.

This system had three major drawbacks:
1. Since only a small number of animals could be housed in each box, housing more than just a few animals became very labor intensive.
2. The animals would sit in stagnant dirty water overnight. This posed a major health concern.
3. Allocating space for more than just a few boxes was a problem.

The requirements for the new housing system were:
1. It would maintain the animals in an environment that was not only clean, but would reduce the risk of spreading disease.
2. It be inexpensive since it sat idle for 8 to 10 months a year.
3. It was able to be quickly assembled and disassembled for cleaning.
4. It would only occupy a small amount of space while in use and in storage.

The new system design is modular, composed of three main units. The first unit is composed of three plastic storage bins that constitute the primary enclosures for the animals. Each bin is capable of holding at least a dozen medium-size frogs. The second unit is a canister-style pump and filter that can pump over 300 gallons per hour. The third unit is an ultraviolet (UV) sterilizer, which is the key to maintaining the health of the animals.

The pump, the UV sterilizer, and the shut-off valves were purchased at a pet supply center. All of the other hardware, including the plastic storage bins, were purchased at a local home and garden center. The total cost of all the parts, including a spare UV bulb and extra filter media, was under $500.

The benefits of the new system exceeded our expectations. The system set-up, along with the break-down and cleaning, is easy and requires little time. The storage requirements are minimal. The design enables us to drain, remove and replace a cage without having to shut the system off. It is possible to change up to 80% of the water in <5 min. without shutting off the system.

The most significant benefit was that the Principle Investigators reported to us that the animals are healthier and consequently surpass their expectations during the procedures. We have also received positive comments by the regulatory agencies.

In conclusion, we have greatly improved the housing conditions of the amphibians as well as increased the efficiency of our housekeeping, for a relatively small investment in time and money.

P127 An Overview of a Ferret Enrichment Program

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Our ferret environmental enrichment program includes various species-specific toys, hammocks and PVC tunnels within the cages. We recently added “playtime” to the environmental enrichment program in which all ferrets are given free access within the animal room for approximately one hour per day. Group activity playtime is supervised by a laboratory animal technician and occurs during the normal husbandry routine. Our ferrets have access to mazes, empty feed bags, and numerous toys that are scattered around the room floor. The ferrets appear to enjoy climbing utility carts to gain access to higher levels, so a custom designed sliding board was constructed of Plexiglas to accommodate their innate climbing behavior. Their naturally inquisitive
nature, along with their ability to run and climb, has increased conspecific interactions within our colony. These additional activities provided to the ferrets on a daily basis have enhanced their environment. In addition, observations of the social interactions between the ferrets have had a positive effect on both the animals and the animal care staff, and have made the ferrets more tractable when handling for experimental procedures.

P128 Customizing Sentinel Rodent Disease Surveillance Programs with Cost-Efficiency and Improved Sensitivity

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We have recently refined our sentinel rodent program to meet investigator needs and allow for prompt recognition and follow-up of pathogens through a sensitive and cost-efficient approach. Research mice are housed in microisolator cages (42 cages/rack-side). Each rack-side is provided a sentinel cage containing two female Crl:CD-1 (ICR)/BR mice received at 3-4 weeks old following dedicated truck delivery. An additional mouse (designated trans-sentinel mouse, TSM) is ear punch-identified and rotated at specific time intervals between 2-3 sentinel cages per room, providing direct exposure for the TSM sentinel across racks totaling 126 cages. All mice from approved vendors arriving at this institution are unpacked aseptically within HEPA-filtered cabinets in a receiving room outside of animal facility corridors. Two mice from each box of sentinels are used for parasitology and comprehensive serology to verify initial health status upon arrival. Schedule maps placed in rooms instruct technicians which rows per rack are to provide dirty bedding sentinels during weekly cage change-outs, including a dirty cage and used water bottle, to ensure exposure of all sentinels to adequate volumes of waste material at least twice per diagnostic test interval. Quarterly testing alternates between clinical and comprehensive serology panels, plus parasitology, from all fixed-position sentinels. TSMs provide for whole-animal health comprehensive monitoring every quarter, thereby abbreviating the time for response to less common agents while containing costs. One of the two fixed-position sentinels is also sent for whole-animal monitoring during the alternate quarters (when no TSMs exist), after which all animals are replaced (± 8 months of age). Cage mates and hold-back serum specimens allow for follow-up testing in the case of suspect or indeterminate results. The resulting sample size per test cycle is a minimum ratio of 6 per 126 cages for serology panel assays. Probability statistics demonstrate benefits of this scheme compared to alternative approaches under conditions of varying prevalence and test sensitivity and specificity.

P129 Novel and Economical Structural Enrichment for a Unique Colony of Group-Housed Macaques: Successes and Failures

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It is our intent to offer our macaques (fascicularis and mulatta) an opportunity to express species-typical activity in a social group when not on study; therefore, we constructed a unique indoor group-housing facility. In November of 2000 the enclosures were complete but no consideration had yet been given to any type of enrichment other than the animals themselves. It is widely accepted that nonhuman primates thrive in a social environment. Victor Reinhardt has published many papers on the success of pair/social housing of macaques and the increased psychological well-being that comes with it. In order to ease the transition from singly-caged housing to group housing we began to design different types of enrichment devices for our animals. Social enrichment provided by group mates seems to overwhelm the effects of inanimate enrichment. With this thought in mind, rather than provide traditional puzzle or chewing devices, we designed structural enrichment. Different levels of perching and visual barriers were installed to allow submissive NHPs to retreat from more dominant and aggressive ones. The animals responded well to all the structures with very little fighting and injuries; however, the structures themselves did not hold up to the animals. Modifications were made to all structures including the removal of all steel chain in the units, the addition of tunnels for novel routes of travel and escape, and monkey towers made from scrap spoons from a rubber manufacturer next door. These economically constructed changes increased the activity in the units and decreased the amount of injuries caused by fighting and trauma caused by the manipulation of the structural devices.

P130 Rodent Transport System for Maintenance of Biocontainment during Radiographic Studies

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We report the use of a polycarbonate biohazard transport box for safe transport and radiology of infected rodents at a radiology suite located outside of the biosafety level three (BSL3) facility. In our facility, Syrian hamsters (Mesocricetus auratus) and mice (Mus musculus) are used as animal models for the study of transmissible spongiform encephalopathies. These studies are conducted under BSL3 containment. Because of the slow development of disease, animals are routinely maintained for 18-24 months before disease onset or natural death occurs. In these chronic studies we occasionally observe conditions such as distended abdomens and palpable masses, where radiology would be useful in clinical decision-making. Our challenge was to identify a method to safely transport and radiograph infected animals using diagnostic equipment located outside of the BSL3 suite, while protecting personnel from exposure and minimizing stress for the often compromised animal. To evaluate the suitability of the box for our purpose, we first calculated the amount of time an animal could comfortably remain in this airtight system. Once the suitability of the transport box was confirmed, a hamster was anesthetized with pentobarbital (50mg/kg). The anesthetized animal was then safely secured to the bottom of the box. Next the transport box was sealed, exterior decontaminated, and carried to the radiology suite. We have successfully radiographed both hard and soft tissue structures using this method. This rodent transport box maintains biocontainment, is radiolucent, and is inexpensive enough to be disposable after a single use.

P131 Innovative Housing and Environmental Enrichment for Bullfrogs (Rana catesbiana)

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At our AAALAC-accredited university, part of my duties include caring for bullfrogs (Rana catesbiana). They are housed in a room dedicated to aquatic animals. The room light cycle is
from 6 a.m. to 6 p.m., with the room temperature being above 68°F and the water temperature about 63°F. The tank bullfrogs are housed in needs to have water deep enough for them to swim and get exercise but also have enough dry space for them to get completely out of the water for resting. We previously housed them in large flat tubes about 28 × 36 × 36 in. fixed at a 30° angle so that 3 in. of water could sit in the lower half. These were a problem because the position of the drainage spout would not allow for full drainage, and to get the tanks appropriately clean, the frogs would have to be removed. It was also difficult to visually observe the frogs. Doing this at least twice a week caused a mild amount of stress to the frogs that we thought could be avoided by changing the design of the tank. Our environmental enrichment program for the mammals is quite extensive but the bullfrogs have not been a major consideration. We also thought some new housing would be a good start to an enrichment program for the bullfrogs. In our decision to purchase new housing, we took into consideration long-term durability, case of use, aesthetic qualities and bullfrog behavior. Clear Plexiglas tanks, about 28 × 34 × 18 in., with spouts were designed. They are fixed at a 25° angle so that 3 in. of water will sit in the lower half. Because they are clear, our frog-monitoring capabilities are increased; however, this caused some panic in the frogs because the tanks provided no hiding places. To reduce stress to the frogs, we thought giving them a place to hide would help them feel more comfortable. We explored our options, searching for items that would stand up to repeated cleaning in our cage washer, be non-abrasive to the frogs' sensitive outer layer of skin, and be commercially available and cost effective. We were using plastic igloo-shaped houses and half tubes of 12-in. PVC pipes as part of our guinea pig enrichment program. These items fit our criteria and were placed in the tanks with the frogs. The frogs were checked twice daily, and as the weeks progressed we found that several of them would be hiding in the objects, and did not startle as easily when someone entered the room. Over time, they also appeared to find the tops or these objects to be a comfortable place to rest out of the water. We also used a floating lily pad made of dense foam. It does not stand up to repeated washing but, because of its low cost, it can be disposed of when necessary. The bulldogs tend to use this often by floating on top of it or hiding under it in the water. The frogs seem to use the entire cage for both swimming in the water and resting on the dry area while hiding and exposed. There has been a significant decrease in the bullfrog mortality rate and the overall appearance of the frogs has improved. All three enrichment options served their purpose and now play a major role in our aquatic enrichment program.

P132 Autocrine Production of IFN-γ by Macrophages Controls Their Recruitment to MRL-lpr Mouse Kidney

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MRL-lpr mice are characterized by production of anti-DNA autoantibodies that trigger glomerulonephritis and death in these mice. Nevertheless, glomerular anti-DNA autoantibody deposits do not always result in glomerulonephritis development. Since IFN-γ+/- MRL-lpr mice produce high anti-DNA antibody levels but are protected from kidney disease, we used these mice to study the events contributing to the kidney inflammatory and destructive processes. Analysis of IFN-γ+/- mice showed defective macrophage recruitment to the kidney that was not caused by decreased levels of MCP-1, a chemokine controlling macrophage migration to MRL-lpr mouse kidney. Instead, impaired IFN-γ production greatly affected expression of ICAM-1 and VCAM-1 adhesion molecules. Transfer of wild type monocytes/macrophages to anti-DNA antibody-free IFN-γ-/- mice did not induce autoantibody production, but provoked upregulation of adhesion molecules as well as a notable infiltration of macrophages to the kidney interstitium, indicating that the source of IFN-γ contributing to local inflammation is not kidney-resident cells, but infiltrating macrophages. Equivalent transfer of T cells to IFN-γ-/- mice did not result in kidney inflammation, further confirming that macrophage-produced IFN-γ is essential for this process. Our findings demonstrate that, independently of autoantibody deposition, autocrine IFN-γ production by infiltrating macrophages is responsible for adhesion molecule upregulation, macrophage accumulation and propagation of inflammation in the kidney. These results outline the pathway of the inflammatory process in lupus and may be useful in designing treatment for disease, even after autoantibody development.

P133 nackt: A Mouse Mutation with CD4 T-Cell Deficiency Associated with Impaired Hair Follicle Cycling and Defective Epidermal Differentiation

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We have previously described an autosomal recessive mutation named nackt (nkt) exhibiting partial alopecia with CD4+ T-cell deficiency. Recently, we reported that nkt (now Cstlnkt) comprises a deletion in the cathepsin L (CtsL) gene. Original efforts using nkt mice have been concentrated on the immunological phenotype; the current study focuses on the dermatological aspects of the mutation. For studies of hair follicle (HF) morphogenesis and cycling, groups of three male and three female at 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30 and 180 days postpartum from nkt/nkt and +/nkt littermates (BALB/c and C57BL/6 backgrounds) were used. Careful histological analysis of skin development of nkt mice revealed a delayed HF morphogenesis and late onset of the first catagen stage. The skin of Cstlnkt+/Cstlnkt mice showed hyperplasia of the sebaceous glands along with structural alterations and abnormal distribution of HFs. Focal areas of keratin 6 expression in the interfollicular epidermis were associated with mild hyperproliferative skin in the mutant mice, suggesting that the normal biology of keratinocytes is altered. Also, we observed overexpression of profilaggrin/filaggrin in the epidermis of nkt mice, suggesting the involvement of CTSL in the processing of this protein. Severe epidermal hyperplasia, acanthosis, and hyperkeratosis were only observed in mice maintained in contaminated environments. The analysis of BALB/c-Rag2-/- Cstlnkt+/Cstlnkt mice (skin collection at 2, 3 and 6 weeks of age from three males and three females) indicates that the skin defect remains under the absence of T and B cells. We describe for the first time the pathology of the nkt skin and provide further evidence in vivo that the lysosomal cysteine protease CTSL plays a critical role in HF morphogenesis and cycling as well as epidermal differentiation. This animal model provides a tool for understanding the role of CTSL in normal epidermal function.
In this study we have demonstrated the effect of diabetes under the development of Schistosoma mansoni worms in diabetes-prone non-obese diabetic (NOD) mice, model of human diabetes type 1. Female and male NOD mice were infected with 10 cercariae from Schistosoma mansoni, BH strain, kept on Biomphalaria glabrata snails. SPF NOD mice were maintained in flexible PVC isolators with positive pressure. The level of urinary glucose was detected by colorimetric method Self-Stik and the results expressed in mg/dl. Seven weeks after infection, the animals were perfunded and adult worms prepared to the measurements. The length of worms was determined in triplicates. The results were analysed according the glucose level at the end of infection. Tukey’s test was used to compare diabetic and non-diabetic groups, with 5% significance level. The statistical analysis showed the influence of the diabetic status of the host and the level of glucose on the development of adult worm. When the infected diabetic female became pregnant, there were no difference in the length of the worms compared to the non-diabetic control. The autoimmune process involved in diabetes concerning the helminth development is showed mainly when glucose level in urine is high.

Anti-idiotypic antibodies are a new type of useful tool for the possible treatment of cancer patients, since some act as antigen-specific immunomodulators. Anti-idiotypic monoclonal antibody (anti-Id MAb) 1E10 (Ab2) was obtained against an Anti-NeuGc-Containing Ganglioside Monoclonal Antibody (Ab1). Vaccination with 1E10 anti-Id Ab has resulted in suppression of tumor growth in mouse systems, suggesting that anti-Id Abs could induce not only anti-anti-Id Abs but also cellular responses to tumor-associated antigens. The main objective of our study was to analyze the lungs’ response of hosts in a syngeneic mouse system by anti-Id MAb, and to compare the difference between the effects of irrelevant IgG or no treatment in an attempt to characterize the antitumor mechanism of 1E10 anti-Id MAb. Females C57Bl/6 mice injected i.v. with B16 melanoma cells were employed. Intravenous administration of 10 µg of 1E10 antibody, ior C5 monoclonal antibody or PBS were realized 10 days after inoculation of B16 cells. Number of metastasis, mitosis/apoptosis relation and histologic changes were determinate in lungs from all groups. The number of experimental metastases was dramatically reduced, while apoptotic cell death was increased in mice treated with anti-Id Ab in comparison with mice treated with an irrelevant IgG or not treated mice. Histologic changes due to anti-Id Ab administration were characterized by abundant round cells infiltration: macrophages and plasmatic cells. The present data suggest that this anti-Id MAb activate more than one of antitumor response against melanoma tumor cells in C57Bl/6 mice lungs.

Mice with severe combined immune deficiency (SCID) unable to produce both B and T cells due a mutation at chromosome 16 (Bosma 1985), are largely used in immunobiological research. However, the occurrence of “leaky” phenotypes may make interpretation of results difficult, and for this reason the frequency of this occurrence in a colony should be determined. The evaluation of the immunoglobulin level by immunobloting test in the mice strain C.B-17scid/Uni started in 1991 at CEMIB/Unicamp The foundation colony was established by hysterectomy derivation of certified breeding pairs received from Pasteur Institute. Since then, this colony is kept in plastic isolators and it is self-perpetuated by ICLAS. To keep this unique excellence pattern, it is important for the center to be able to guarantee the integrity of its mouse and rat colonies, protecting these animals from eventual accidents, such as contamination, mutation, fire, etc. One of the most important ways to keep the quality and integrity of these strains is the cryopreservation of embryos in different stages of development. The Cryopreservation Laboratory accomplishes tasks. The pattern procedure is to obtain and collect 1e-cell stage embryos. The different media and solutions prepared obeyed the methodologies described by Hogan et al. (1986). The two-step freezing technique followed by the maintenance of the embryos in containers with liquid nitrogen was installed in routine as described by Hesrich & Reetz (1988). Now we maintain samples of the different strains cryopreserved such as SPF animals, virus-free animals, mutants, recombinants, etc. We also prepare vasectomized males, lately mating them with ordinary females to guarantee the surgery result. These vasectomized animals are then mated with receptor females, so that they are prepared to receive the embryos that were collected from the donors. Through this technique we make sure that the cryopreserved embryos are viable, obtaining an average efficiency rate of 85%. Through the embryo’s manipulation and washing in media, the laboratory can remove possible pathogens from the embryo, obtaining SPF animals. Lately we had positive results with embryos from the strain BALB/c that were cryopreserved six years ago, showing that the routines installed are working according to the literature. The next step will be the utilization of this background for the development of new animal models, such as transgensics and knockouts.

Mice Reared in Plastic Isolators

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CEMIB is the only institution in Latin America that maintains foreign, imported and certified mice and rats strains on its foundation colony. It is also one of the five institutions acknowledged by ICLAS. To keep this unique excellence pattern, it is important for the center to be able to guarantee the integrity of its mouse and rat colonies, protecting these animals from eventual accidents, such as contamination, mutation, fire, etc. One of the most important ways to keep the quality and integrity of these strains is the cryopreservation of embryos in different stages of development. The Cryopreservation Laboratory accomplishes this task. The pattern procedure is to obtain and collect 1e-cell stage embryos. The different media and solutions prepared obeyed the methodologies described by Hogan et al. (1986). The two-step freezing technique followed by the maintenance of the embryos in containers with liquid nitrogen was installed in routine as described by Hesrich & Reetz (1988). Now we maintain samples of the different strains cryopreserved such as SPF animals, virus-free animals, mutants, recombinants, etc. We also prepare vasectomized males, lately mating them with ordinary females to guarantee the surgery result. These vasectomized animals are then mated with receptor females, so that they are prepared to receive the embryos that were collected from the donors. Through this technique we make sure that the cryopreserved embryos are viable, obtaining an average efficiency rate of 85%. Through the embryo’s manipulation and washing in media, the laboratory can remove possible pathogens from the embryo, obtaining SPF animals. Lately we had positive results with embryos from the strain BALB/c that were cryopreserved six years ago, showing that the routines installed are working according to the literature. The next step will be the utilization of this background for the development of new animal models, such as transgensics and knockouts.

CEMIB Multidisciplinar Center for Biological Investigation, UNICAMP—State University of Campinas, Campinas, São Paulo, Brazil
tion indicating that leakage is age-dependent. On the last profile taken at CEMIB/Unicamp, using SCID older than 40 weeks, the leaky frequency was 9%. The result is according to the literature and described in aging of the SCID mice.

P138 The Effect of the Peptides Isolates from Casein Hidrolisates on Arterial Blood Pressure in Wistar Rats

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Peptides that display biologic activities have been identified from different sources such as casein, snake venom, etc. The activity and the mechanism of action from two casein hidrolisates peptides (YPVQPFTE e INKKI), were characterized. Arterial blood pressure recording analyses the intravenous injection of the peptides and its effects on bradykinin (BK), angiotensin I (AI) and difenidramine (DFH) administration. The carotid artery of adult male Wistar rats, anaesthetized with chloral hydrate, was registered using a STE systems polygraph (10mm/min paper speed and 0-120 mmHg calibration scale) coupled to a physiological pressure transducer. Hypotensive areas resulted from intravenous injection were obtained by integrating the recording drawn with a scanner and comparing with peptides effects. The peptide YPVQPFTE (30µg/kg) do not display a intrinsic activity, but potenciated the hipotensive action under BK (0.5ng/IV) and its action do not alter the Angiotensin I to II conversion, suggesting that the effect was not relationship with Angiotensin Converting Enzym. The peptide INKKI (75µg/kg), presented a hypotensive effect but not potenciated the BK action. This peptide presents homology with mastoparanos and its actin would be under histamine delivery. This hipotesis was confirmed when the its effect was blocked animals were treated with DFH.

P139 Congenic Fischer (F344) Rat Carrying Lymphopenia (Lyp) Gene from BB Rat

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Diabetes-prone (DP) BB rats are born with the peripheral T cell (lymphopenia) linked to the Lyphem gene on chromosome 4. The clinical onset of diabetes develop in a narrow window of 50-70 days of age in BB Lyp/Lyp rats that are MHC RT1B u/u on chromosome 20. Congenic F344.Lyp rats were generated by intersgression of the Lyp gene onto diabetes resistant MHC RT1B Lyp/Lyp rats to test the hypothesis that this combination of Lyp and MHC would induce organ-specific autoimmunity other than type 1 diabetes. A speed congenic line was generated by marker-assisted selection in 5 cycles of cross intercross breeding with F344 congenic panel. The peripheral blood showed a significant reduction in the total number of leucocytes due to a marked reduction of lymphocytes. Cell surface staining of lymphocytes subsets were performed at 150-180 days of age. The following antibody panel were selected for phenotypic flow cytometric analyses of T-cell subsets: CD4 (OX-35), CD8α (OX-8), CD3 (G4.18), alpha-beta TCR (R73), and CD90 (OX-7). Collection of lymphocytes from blood, thymus, spleen, and lymph node revealed significant differences between the F344.Lyp/Lyp and +/- genotypes.

The major findings were the peripheral lymphoid organs showed a marked decrease in CD4 and CD8 positive cells. Our conclusion is that the lymphopenia phenotype was rescued in the congenic line by introgression of the Lyp mutation from the congenic DR.Lyp/Lyp rat.

P140 Age-Related Differences in Behavioral and Neurochemical Parameters after Unilateral Neostriatal Injections of 6-hydroxydopamine in Rats: A Better Approach to Studying Parkinson’s Disease?

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Of the many models for Parkinson’s disease, the 6-hydroxydopamine (6-OHDA) model is one of the better known. Usually the experiments use young adult rats; however, since Parkinson’s disease has been related to age, it is important to analyze the relevance of this factor in the model. In this study, the behavioral and neurochemical effects of unilateral neostriatal injections of 6-hydroxydopamine were analyzed in male Wistar rats from two age groups: middle-aged (1 year, n = 22) and young (9 weeks old, n = 14). The control group received two unilateral injections of the vehicle (9% saline and 0.2% ascorbic acid) in two different regions of neostriatum; the experimental group received two 6-OHDA injections (10 µg/4 µl each) in the same regions. All rats were treated for spontaneous behavior in the open field (60 x 60 x 40 cm) on days 1, 3, 5, 7, 11, 15, 19, 23 and 30 after the surgery; each behavioral test lasted 15 min. and included turning, scanning and locomotion. After day 30, the animals were euthanized and their brains removed for neurochemical analysis with High Performance Liquid Chromatography with Electrochemical Detection.

Many differences were observed between the two age groups. The middle-aged rats were more sensitive to the toxin; they showed bigger dopamine depletions in both neostriatal regions (medial and lateral) than the young rats, and their metabolisms were also different. Both the middle-aged and young rats showed characteristic ipsilateral asymmetry in turning and scanning behavior, but the time course and the tendencies were different in both age groups. The results of this study demonstrate age-dependent neurochemical and behavioral differences after unilateral neostriatal lesion that could be important in better understanding the progression and pathology of Parkinson’s disease, and should be taken into account in similar studies.

P141 Production and Maintenance of Transgenic Mice (Mus musculus) B6D2F1/J for a Research Project

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The production of transgenic mice is a very common practice not only in developed countries, but also in underdeveloped countries where they are required for research. Direct microinjection of cloned ADN fragments in the embryo prounucleus (fertilized egg) is the method most used and one of more successful in the production of transgenic animals. Since these animals are very valuable for research, the principal objective of this study was to learn the reproductive parameters of B6D2F1/J mice and compare with other sources to ensure that the animals have a good breeding performance. The second objective was to ascertain how many animals have the gene SERCAZ-Luc (heart sarcoplasmic reticulum) and how many expressed this gene. Western Blot Technique was used to learn the gene expression. This information is very important to support the research project that has used these animals. Mice came from Anderson Center of Houston Univer-
The animals were housed in microisolators and were fed ad libitum with Pico Lab Rodent Diet. F1 generation was weighed every week until 100 days old to determine the growth line; other reproductive parameters were also evaluated. The preliminary results indicated that all the reproductive parameters were similar to other sources (Jackson Laboratories and HSD). Early identification of the gene expression is important in avoiding overproduction of transgenic mice.

**P142 Survival of Leydig Cells under the Protection of Zinc in Fischer Rats (Rattus norvegicus) Treated with CdCl2**

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The role of zinc in investigating mechanisms to prevent cadmium carcinogenesis in the rat testis is not clear. In rodent testes, cadmium induces severe necrosis, followed by chronic degeneration. A single dose produces a high incidence of Leydig cell tumors. The mechanism to avoid the death of Leydig cells is unknown, but putative feedback increased luteinizing hormone (LH) production due to low circulating androgen has been implicated in causation of proliferative lesions within degenerate, hypofunctioning testes. Thus, the role of the zinc to prevent the toxicity of cadmium in Leydig cells in Fischer rats was studied. Four groups of 6-month-old rats (n = 44) were used. One group received 20 µmol CdCl2/kg s.c. weekly for 5 weeks (total dose 100 µmol/kg). The second group was given 20 µmol CdCl2/kg s.c. and 1 mmol of zinc acetate s.c. weekly for 5 weeks (total dose 100 µmol/kg and 5 mmol/kg, respectively). The third group was given 1 mmol of zinc acetate s.c. weekly for five weeks. The last group was given saline solution s.c. for 5 weeks. All the animals were euthanized 8 months later. The number of Leydig cells killed was higher in the cadmium group (82.48%) than in the cadmium-zinc group (67.74%) and the zinc group (11.52%) in relation with the control group. The cadmium zinc minimizes the toxic effects of cadmium on Leydig cells, and consequently the Leydig cells produced enough testosterone to avoid the increase of LH levels.

**P143 Animal Model of Oxidative Stress Induced by Hemolysis**

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Oxidative stress may appear from different sources, inducing an imbalance between the antioxidant defenses of the organism and the oxidant and pro-oxidant agents, which results in cell damage. Several studies connect oxidative stress to chronic illnesses such as atherosclerosis, diabetes, Alzheimer’s disease, and others. The hemolysis of the erythrocytes release pro-oxidant agents (hemoglobin and lipo-oxygenase-like enzymes) to the circulatory stream, increasing the production of reactive oxygen species. Accordingly, establishing new animal models of oxidative stress will be useful to study the effect of oxidative stress on the origin and development of several pathologies.

The aim of this work was to induce oxidative stress by hemolysis, and then to determine the levels of end products of oxidation of fatty acids in plasma and other tissues. Male Sprague Dawley rats weighing 300 ± 20 g were maintained at 22 ± 2°C, 50-55% r.h., with a 12 h light/dark cycle, fed ad libitum with free access to tap water. The animal were divided in two groups (4 rats/group): Control (no intervention) and experimental group (40 mg/kg acetylphenylhydrazine i.p.). After the injection, no external signs of pain were observed. Acetylphenylhydrazine induces a self-limited hemolytic anemia in rats. Four days after injection, the animals were beheaded, the blood immediately collected and centrifuged, and the plasma obtained. The adipose tissue, liver, kidney, vastus lateralis muscle, heart, and brain were sampled in the same order, immediately frozen in liquid nitrogen and stored at −70°C until analysis. The malondialdehyde levels were determined in plasma and tissues as being a biomarker of oxidative stress. A significant increase of malondialdehyde levels in plasma has been obtained, showing that oxidative stress has taken place. However, no changes have been observed in the tissues, showing that there are enough mechanisms of protection against oxidative stress induced by hemolysis in tissues.

**P144 El Enriquecimiento Ambiental como un Factor Clave en los Modelos Desesperanza Conductual para Estudio de la Depresión**

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Dos de los modelos más importantes para el estudio de la desesperanza conductual son los “modelos de Estrés psicosocial” de “Estrés suave, crónico e imprevisible.” Ambos poseen una elevada validez predictiva porque relejan eficientemente los efectos de determinados tratamientos farmacológicos sobre una condición humana simulada (Depresión). Además, se pueden ensayar potenciales psicofármacos con un mínimo posible de falsos positivos o negativos. Ambos modelos presentan consistencia teórica con la condición humana que pretenden medir. Éstos modelos, inducen un estado de desesperanza conductual a través de alteraciones en la temperatura ambiental, cambios de los compañeros de caja, variaciones en las dimensiones de las cubetas, deprivación de agua y alimento, entre otros. Esta descripción de los procedimientos de ambos modelos supone una alteración sistemática de los parámetros de enriquecimiento ambiental. Algunos de los indicadores más importantes que presentan las ratas alojadas en ambientes empobrecidos son: Inmunosupresión, incremento de la cortisona en plasma, pobre reactividad a la estimulación ambiental, pérdida de peso, decremento en los niveles de dopamina y por tanto presencia de estados ahedónicos. Éstos indicadores y otros como la reducción de los patrones de automantenimiento, defensa y reproducción son los mismos utilizados para evaluar la desesperanza conductual. Esta consistencia entre empobrecimiento ambiental y desesperanza, permitiría validar concurrentemente ambas condiciones para obtener un modelo heurísticamente potente para el estudio de la depresión. La investigación con humanos ha resaltado el papel que desempeña la estimulación temprana y el bienestar social como factores que reducen la probabilidad de presentar un episodio depresivo. La investigación en este sentido podrá sugerir una redefinición etiológica de los modelos animales para el estudio de la depresión.
P145 Development of Biomodels for Haemophilus influenzae Type B Infection

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The epidemiological situation of diseases caused by Haemophilus influenzae type b is increasingly dramatic, and is considered a global human health problem. There are several animal models to reproduce different aspects of the clinical forms of Haemophilus influenzae infection, among them inbred mice, rats and guinea pigs. The aim of the present work was integrating models established in our institute to answer several questions regarding the experimental infection by Haemophilus influenzae and exploring their potential usefulness. C57/BL6, CBA/j and BALB/c inbred mice strains; infant (5-7 days old) Sprague Dawley rats; adult (400-450 g) Duncan Hartley guinea pigs and the Eagan strain of Haemophilus influenzae were used. Infant rats were inoculated by nasal or intraperitoneal routes, and mice and guinea pigs were treated with virulence enhancement substances for Haemophilus influenzae infection. The disease was successfully reproduced in the three laboratory animal species assayed. However, infant rats were better for studies of bacteremia, inbred mice for efficacy tests using death rate as endpoint, and guinea pigs to study the pathogenesis of the disease. As novel results, we demonstrated the efficacy of trypsin as virulence enhancement agent and the development of Haemophilus influenzae infection in guinea pigs. This system of biomodels could be a valuable tool for understanding pathogenic aspects of Haemophilus influenzae infection and studying new products against them.

P146 The Animal Ethics Committees for Care and Use of Animals in Research, Teaching and Experimentation in Costa Rica

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In the frame of Law #7451, “Animal Welfare,” that was promulgated in Costa Rica since 1994, the first Ethical Committee on the animal experimentation within the University of Costa Rica was created in 1998. This committee was called “Institutional Committee for the Care and Use of the Animals (CICUA).” The University of Costa Rica uses the largest amount of laboratory animals in the country for teaching and scientific purposes; therefore, the CICUA has the responsibility of fulfilling the requirements of the legislation and must keep watch over all scientific and technological activities that use animals or their derivatives within the University of Costa Rica. The CICUA must clarify, interpret, review, and evaluate the institutional programs of tests, investigation and teaching activities related to the care and use of the animals. The composition of the CICUA is determined by Law #7451 and has as its members representatives from each school that uses animals in teaching or in experimentation within the university, a representative from Laboratory Biological Assays (LEBi) who coordinates this committee, other institutes and an external member that must belong to ACCMAL. Before proceeding with any project involving animals, the individual researcher must gain approval form the CICUA, who reports the agreement of committee to the higher authority in the university. The higher authority must then keep watch over all scientific and technological activities that use animals or their derivatives within the University of Costa Rica. The CICUA must clarify, interpret, review, and evaluate the institutional programs of tests, investigation and teaching activities related to the care and use of the animals.

P147 Regulation of Animal Experimentation in Costa Rica

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The first law in Costa Rica governing animal experimentation was published on December 13, 1994. It is known as Law #7451: “Animal Welfare.” Articles 3, 10, 11, 12, 13 and 21 of this law describe the basic conditions for animal welfare, the basic requirements for conducting experiments with animals, the use of alternative methods, and the supervisory function of the Ministry of Science and Technology (MICIT), among other things. The sanctions that prevail on the breach of this law are also discussed. The regulation of these articles was elaborated by a work group composed of representatives from ACCMAL (Central American, Mexican and Caribbean Laboratory Animal Science Association), the World Society for the Protection of Animal (WSPA) and the MICIT. Along with this law, in 1998 the Guide for the Care and Use of Laboratory Animals and the “Formularies for the Inscription of Experiments with Animals” were published. At the same time, the National Technical Committee (CTN), which is responsible for advising government on matters relating to animal welfare and the use of animals in research, teaching and experimentation in accordance with the national legislation, was created. The CTN is responsible for the creation of Institutional Animal Care and Use Committees (ethical committees) in all institutions that conduct animal experiments in the country. The diffusion of the content of the law through different advanced training courses organized by the University of Costa Rica and ACCMAL, with the support of other organizations like ICLAS, SECAL, AMCAL, has promoted the responsibility of reduction, refinement and replacement of animals for scientific and teaching purposes.

P148 Evaluation of the Laboratory Animal Technology Program at the University of Buenos Aires

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The three-year Animal Care Technology program was created at the University of Buenos Aires in 1988. The program syllabus comprises courses related to biological and exact sciences, including a course on Animal Care Technology. The program has a very important technological component that is taught with increasing depth over six semesters in order to develop knowledge of the common laboratory animals species and the principles and practice of their care, maintenance and use. The “3 Rs” (replacement, reduction and refinement) are constantly strengthened. The program seeks to prepare technologists to take responsibility for the everyday management of the animal house and assist in research laboratories; for this objective, the consultation of books, scientific journals and the Internet is a must. Another objective of the program is to prepare these technologists to work in both developed and developing countries where the scarcity of human and material resources requires special abilities to develop initiatives. The teaching methodology is based on the integration of commissions assigned to one student from each semester of the program, for a total of six students and one assistant. Each commission involves all the tasks related to animal housing and well-being; over
In conclusion, three fundamental reasons have shown the necessity of this program:

1. The effective contact (refinement) with the animal in the learning process of the technologist translates into non-stressed animals.
2. The reduction of the number of animals used.
3. The opening of a new range of working possibilities benefits low-income students, which is especially important for South American countries.

P149 IACUC Evaluation for Future AAALAC, International Accreditation

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In the last five years, the laboratory animal science program at CINVESTAV has undergone great development. National and international rules, such as NOM-062-ZOO-1999 and the Guide for the Care and Use of Laboratory Animals, have provided guidelines to follow, and the IACUC has established the goal of following those guidelines to obtain AAALAC certification for our Laboratory Animal Production and Experimental Units (LAPEU) Program. For evaluation purposes, the LAPEU had to fulfill and inform the established standards in the Standard Program Description:

- Institutional policies and responsibilities;
- Animal environment, housing and management;
- Veterinary medical care and personnel qualifications;
- Physical plant;
- Control and diagnostic laboratory evaluation of the quality of animals, equipment and facilities;
- Monitoring of the care and use of animals; and
- Occupational health and safety.

After the evaluation of the steps above, the IACUC will be able to assess CINVESTAV’s ability to achieve AAALAC accreditation.

P150 Handbook of Surgical Care in Rodents

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If appropriate surgery techniques are not used or if animal welfare is neglected before, during or after surgery, the health of the animals and the performance of the research project are put at risk. People involved in lab animal science must be knowledgeable in physiology, pharmacology and anatomy to achieve good results in their research. This handbook serves as a resource of information pertaining to the care of laboratory animals in our animal facility before, during and after surgery. Information was collected from many sources (books, handbooks and Internet information) to ensure that the topics covered in the handbook are up to date. The chapters are: Standards in Laboratory Animal Surgery; Surgical Facilities; Surgical Instruments and Materials; The Importance of Laboratory Animal Care Before, During and After Surgery; The Importance of Anesthetics; and The Importance of Analgesics. The handbook also includes a glossary, a bibliography and a list of web references. The illustrations reinforce its content, and the tables provide additional information on species characteristics and breeding data, euthanasia techniques, needle sizes and recommended injection volumes, etc. Personnel in our animal facility find this handbook a useful reference.

P151 Trends in the Use of Laboratory Animals in Colombia

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This paper discusses trends in the use of laboratory animals in Colombia over the last ten years. The National Health Institute is the national reference center of laboratory animals; it has animal production facilities that provide animals to the rest of the scientific centers of the country. The animals are produced and housed in accordance with international recommendations; public health barriers ensure the quality of produced biomodels and containment is achieved by use of a ventilation system and autoclaving all food, equipment and clothing items that are used. A Committee of Ethics evaluates the performance of the ethics principle regarding the animals and ensures that they are treated humanely; decisions regarding euthanasia are made according to AVMA guidelines.

Until 1991, the Swiss mouse was traditionally used for research; little importance was given to the production of laboratory animals. However, after the new professional personnel attended a series of training courses, and due to the need for improved quality in experimental models, the use of other species, colonies and strains became necessary. Certified genetic banks were imported, and little by little, inbred strains and outbred colonies. Today, most of the animals (89%) are used in making and controlling biological products; outbred ICR and NIH mice are typically used for this purpose. B/ALB/Cand C57BL/6 mice (4%) are used in microbiology, genetics and parasitology investigations. Hartley guinea pigs (3%) are used in the production and control of biological and molecular physiology and cellular biology investigations. Mongolian gerbils (2%) are used in studies of Giardia. Wistar rats (1%) are used in neuroscience, genetics and nutrition investigations; Golden hamsters (1%) are used in entomology and virology studies.

P152 Manual de Genética de Roedores de Laboratorio: Principios Básicos y Aplicaciones

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Este resumen es la presentación en sociedad de nuestro libro sobre genética de roedores de laboratorio. Se trata del primer libro en idioma español dedicado a esta temática y por lo tanto creemos que será una herramienta útil para el entrenamiento de estudiantes, personal técnico y profesionales en los aspectos de la genética de animales de laboratorio, particularmente en España y Latinoamérica. Los contenidos del libro abarcan un amplio espectro de la genética del ratón, la rata, el hámster, el cobayo y el jerbo de laboratorio. El capítulo I realiza una revisión de conceptos de la genética de los mamíferos necesarios para comprender el resto del libro. Incluye temas como la organización, estructura y replicación del ADN, las características del genoma, y las herramientas para el análisis del ADN al nivel molecular (incluye una sección de citogenética del ratón). El Capítulo II hace una recorrida por la biología del ratón y su manejo reproductivo, incluyendo una revisión de las técnicas de reproducción asistida y criopreservación en el ratón. En el Capítulo III encontramos los conceptos más importantes acerca de la sistemática de los roedores de laboratorio y el uso de líneas de origen salvaje para la
In order to prepare the process, CONEVEt offers a guide for related with laboratory animal science is strongly recommended. This includes characterizations, uses and nomenclature of the lines consanguineous, cosignéicas, congénicas and congénicas recombinantes, between others. Tras una breve reseña de the genética de la hiato-compatibility and the laws the gobiernan (Capítulo V), el libro presenta una descripción completa and actualizada of the distintos lines genéticas in the ratón of laboratorio (Capítulo VI). Este capítulo describes the distintos tipos of cruzamientos and the marcadores genéticos más utilizados in the construcción of estos maps. El Capítulo VII está dedicado to the mutaciones and sus consecuencias, conteniendo a una revisión actualizada of the sistemas of mutagenesis disponibles en el ratón. El Capítulo VIII revisa in forma detallada los métodos of transgenésis in the ratón of laboratorio, incluyendo la inyección de ADN in el pronúcleo and the mutagenesis dirigida por recombinación homóloga (ratones knock-out y knock-out condicionales). Ya en el final, el Capítulo IX presenta una reseña of the mutaciones of the rata and el ratón usados como modelos of enfermedades humanas, incluyendo enfermedades hereditarias simples (Mendelianas) and complejas (multigénicas). Finalmente, el libro cierra con un capítulo dedicado to the estadística aplicada to the animales of laboratorio. El libro contiene además apéndices with listas of proveedores comerciales and animalarios of España and América Latina, bases of datos and direcciones of Internet relacionadas a the genética of roedores and programas of computación.

P153 Laboratory Animal Medicine Certification Program for Veterinarians

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Laboratory animal medicine is a specialty of veterinary medicine accepted by the National Council for Veterinary Medical Education (CONEVEt) and is a compulsory subject in some veterinary medical schools in Mexico. A new Mexican law, the Norma Oficial Mexicana 062-ZOO-1999, orders that each research unit working with laboratory animal must have a named veterinarian certified in laboratory animal medicine. Therefore, the CONEVEST and the National Council for Evaluation of Higher Education (CENEVAL) have developed, on a voluntary basis, an examination program to evaluate the knowledge and abilities of veterinarians working in the field. The examination process is divided into two independent sections. Veterinarians with less than 10 years’ experience receive a comprehensive examination on: Ethics and Legislation; Husbandry; Management; Handling; Well-being; Laboratory Animal Medicine Including Anesthesia, Aseptic Technique and Surgical Procedures; Euthanasia; Necropsy; and Biosafety. This eight-hour exam is divided into 4 sessions: 1. General examination by a multiple-choice exam (3 h); 2. Clinical competency test (3 h); 3. Necropsy (1 h) and 4. Handling and resting (1 h). Veterinarians with 10 years’ or more experience are examined on a case-by-case CV review, looking for a balanced, integrated and continued education and training on the above-mentioned topics. In addition, publication of scientific work and active or demonstrable relationship to a scientific association directly related with laboratory animal science is strongly recommended. In order to prepare the process, CONEVEt offers a guide for the aspirants with detailed information.

P154 Harmonization and Improved Quality and Well-being of Laboratory Animals in Mexico Via the Norma Oficial Mexicana 062-ZOO-1999

R Hernandez*, O Villanueva, R Cortes, P Santillan


In Mexico, scientific research with laboratory animals is conducted mainly by public universities, national health institutes and public hospitals. Since scientific research is not a priority of those institutions, scarcity of funds, equipment and facilities are common problems for both researchers and animal facility managers. On the other hand, it has been necessary for the local scientific research community to publish in international journals where high standards of laboratory animal care and use are required. In addition, concern for the well-being of the animals used for experimental research has been rising fast in some groups of the society with influence in the congress. Therefore, there was a real necessity to improve the quality and well-being of laboratory animals. For all the above-mentioned reasons, in 1999 a group of scientists and veterinarians decided to promote a legislation from the scientific point of view instead of a legislation promoted by the pressure of antivisesection groups. The Norma Oficial Mexicana 062-ZOO-1999 is a nationwide observance document that must be followed by both public and independent organizations using laboratory animals. The Norma offers guidelines and information on four main areas: 1. Minimal requirements for husbandry, care and use of the most common laboratory animal used in research, 2. Implementation and operation of the Institutional Animal Care and Use Committee, including appointment of the named veterinarian, 3. Analgesia, anesthesia and euthanasia, and 4. Biosafety. The Norma has been in effect since August 2001 and allows institutions five years to meet the required standards. We sincerely believe this is a way to improve quality and well-being of laboratory animals in Mexico as well as comply with international standards.

P155 Strategies for Improving the Effectiveness of National Society of Laboratory Animals on Husbandry and Welfare

J. Martinez*, J Diaz

The Cuban Association for the Care and Use of Laboratory Animals (SCCAL) was created in 1999, and three years ago we saw results for our work on improving husbandry and welfare of laboratory animals.

Five years ago, we researched possible institutions and organizations to partner with in order to increase our effectiveness. We were also looking at the organizations’ technical infrastructures to determine which would best help us promote results.

The better partners have been the National Regulatory Authority and the National Veterinary Science Committee, which provides legal support to SCCAL’s propositions. A national electronic mailing list created by Public Health, INFOMED, has been a useful instrument for us as well.

We have released our National Resolution about laboratory animals for comment and, through the Veterinary Scientific Committee, we will present it to the National Assembly of Power Popular, which has the responsibility to study and proposeprobation of our laws.

We have other examples that explain our results. They can be useful for other countries from Latin America that need to improve the impact of your society on the husbandry and welfare of laboratory animals at a national level.
P156 SCCAL and CECMED: Relation and Results after Three Years

I. Martínez*, J Díaz, I Beausoleil

The Cuban Society for the Laboratory Animal Science (SCCAL) identified the National Regulatory Authority for Human Drugs (CECMED) as one that could give legal support for SCCAL’s objectives. The objectives of the Society and the interest of CECMED coincide on compliance with principles of Good Laboratory Practices for non-clinical laboratories in the pharmaceutical industry. Compliance with these principles is verified during license inspections and laboratory certifications as well as during a comprehensive evaluation of their dossiers. Keep in mind that GLP covers personal components and the experimental system.

One result of this partnership was the guidelines for the animal protection and welfare that we proposed as a reference document for CECMED to use during the inspection process.

These guidelines consider, among other subjects, the constitution and functioning of the Institutional Committee for the ethical use of laboratory animals. The acceptance and application of this document guarantee the compliance of ethical issues, and it clarifies the lab evaluation process for CECMED.

We will also discuss other regulatory activities performed for us that helped improve the welfare and ethical use of laboratory animals in our country.

P157 Online Postgraduate Training on Animal Welfare and Research for Technicians and Researchers

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The aim of this work was to prepare educational materials accessible from the Internet as a method to train technicians (medium- and/or high-level) and postgraduates in biomedical sciences on laboratory animal science, and also to provide an online academic resource on animal welfare and research.

The materials are designed to supplement postgraduate coursework on laboratory animal science; students may consult these multimedia materials as many times as they desire. The online training is complemented by other educational resources, such as videoconferences, electronic mail, and chats.

The team responsible for this educational project is made up of professors and researchers devoted to several fields of laboratory animal science (including pharmacologists, physiologists, and managers of animal facilities) from the University of Granada and the University of the Balearic Islands (Spain). The team receives support and assessment from the Scientific Instrumentation Center of the University of Granada, Spain, mainly for audiovisual resources and image digitalization.

The contents of the online educational materials have been arranged as follows:

I. Course on Animal Research for Researchers
   I. Legislation, ethics, and the 3 Rs
   II. Basic biology and husbandry of relevant laboratory animal species
   III. Physiological needs and welfare of animals and related factors
   IV. Handling and conduct: basic techniques and euthanasia
   V. Anesthesia, analgesia and basic principles of surgery
   VI. Occupational health and safety
   VII. Design of experiments

2. Course on Experimental Methods and Techniques in Physiology and Pharmacology
   I. Introduction
   II. Excitable tissues
   III. Circulatory and respiratory systems
   IV. Digestion, nutrition and urinary system
   V. Endocrine system and reproduction

The web site was designed as an electronic book, arranged by chapters. The text is enriched with links to figures, slide presentations, additional PDF documents, short videos and a glossary. Also, relevant references are listed after each chapter. Links to related web sites providing further information are also shown. Self-evaluation exercises are provided after each chapter and include self-quiz questions, critical thinking questions, schemes and figures.

P158 Presentation on Interactive Educational Programs in Laboratory Animal Science in the Hispanic Community

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The globalization process in which Mexico is immersed, uniform approaches are being developed to regulate the operation of activities related to efficient production, care, handling and use of laboratory animals in research centers, universities and institutions. The hope is to guarantee, insofar as possible, the well-being of the animals used as experimental models. These approaches are based on Mexico’s Official Norm—062-ZOO-1999: Technical Specifications for the Production, Care and Use of Laboratory Animals. One of the main requirements of this law concerns the qualifications and certification of personnel who will work with animals. In México, a formal Educational Program does not exist in Laboratory Animal Science such allow continuous formation of human resources in the use, care and handling of laboratory animals (students, caretakers and investigators). Over way the Autonomous University Juárez of Tabasco, through the Academic Division of Health Sciences (DACS) is designed a solid training Program in Laboratory Animal Science. Such a program is essential to contribute to the humane use and responsible care of the animals, to unify training approaches, and to qualify the users in the techniques and handling of the animals. We visualize the future as process to certify personnel who works in the research facilities.

P159 Special Considerations Associated with Importing Animals from Unapproved Sources

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The global research animal exchange, principally genetically engineered mice, entails many risks. Careful evaluation of source institution and transportation risks must be appropriately countered at the receiving institution to safeguard the resident animal population often numbering in the thousands to tens of thousands. Source institution risk evaluation must include husbandry, facility, and disease management practices. The source institution evaluation should revolve around the congruence of the health status, health history, and management practices with the receiving institution. Transportation risk has the most numerous and variable risk factors, frequently falling outside the sphere of influence of both the source and receiving institution. The arrival of “clean” animals at the receiving institution should be met with scrutiny and suspicion, primarily due the transportation-related risks. Effective management of these clean animals is essential be-
cause this is the only point where the receiving institution can exert control on the research animal exchange process, which is in effect damage control. A major point of conflict is effective damage control versus an investigator’s continued use of these clean animals. This institution-specific, yet customizable plan for meeting a given institution’s animal receiving needs from around the world also facilitates the investigator’s animal-related needs. The plan’s primary goal is to confidently determine the new arrivals’ health status, while providing for their timely introduction into the colony and providing investigators access to the animals.

P160 Past or Present?
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One of the most important elements in the physical and social environment of animals used in biomedical research is the quality of the equipment used in their care. The cage environment needs to be comfortable, safe, and escape-proof, with easy access to food and water. Animals should have adequate ventilation and be kept dry and clean. Cage design should allow for inspection of occupants without disturbing them. Proper housing and management of animal facilities are essential to the well-being of the animals, the quality of the research data, the effectiveness of teaching or testing programs in which animals are used, and the health and safety of personnel.

Cages can be made of plastic, sheet metal, copperplate, sheet steel, wire mesh, acrylic, steel, and polycarbonate. Generally, the caging materials should be sturdy and durable, and have smooth impervious surfaces that meet the needs of the animal while providing sanitation without accumulating dirt, debris, and moisture. Most vivariums in Mexico don’t have good equipment because they lack adequate budgets, so almost all of the cages are broken, the walls are opaque, and the sheet metal is rusted. Any of these conditions could allow the animals to escape or cause accidental entrapment or injury to the animals. Furthermore, it is impossible to observe the animals through the opaque walls with minimal disturbance to them.

A management program has been proposed that provides the environment, housing and care that permit animals to grow, mature, reproduce, and remain healthy, and thus improve the condition of Mexico’s vivariums.

P161 Problematic of the Dermatophytosis Diagnoses in Laboratory Animals
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In Venezuela there exist few published data about the incidence of the superficial mycoses in laboratory animal colonies. The objective of this study was to establish the incidence of dermatophytosis in laboratory animals with cutaneous lesions. Forty-four animals (12 rabbits and 32 guinea pigs) from colonies located in different regions of Venezuela were studied. Epidermal scale samples were taken from each animal, and for the fungal diagnosis direct exams with 10% KOH preparation plus Parker ink were made and then cultivated in Mycosel Agar and Lactrimel Agar with cloramphenicol. The identification of the isolated dermatophytes was accomplished by the macro- and microscopic characteristics of the cultures. Of the 44 animals studied, 38 (86.36%) were positive for fungi. Of the 32 guinea pig samples, 27 (84.4%) were positive: 13 (40.6%) with Microsporum canis, 10 (31.3%) with Trichophyton mentagrophytes and 4 (12.5%) presented infections with both of them. Eleven of the rabbits (91.7%) were positive for Microsporum canis.

A high proportion (26.59%) of infections with dermatophytops was observed in the animals studied. T. mentagrophytes and M. canis were the principal etiological agents isolated and the lesions were located mainly in ear, which would produce a misdiagnosis if lab tests are not taken into consideration.

P162 Evaluation of Two Physical Forms of Commercial Diets for Laboratory Mice and Rats Regarding Reproductive Performance and Weanling Weight Gain

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Results derived from animal experimentation depend to a considerable degree on the health and welfare of the animals employed. This, in turn, reflects the quality of housing, care, and nutrition provided those animals. Most diets designed for a particular species provide a reasonable balance of nutrients in amounts sufficient for normal growth, maintenance, and reproduction. Little information, however, is available on the effects of the form in which the food is offered on the performance of laboratory animals. In order to compare two different physical forms of rodent diet, this trial analyzed reproductive performance and gain weight of mice and rats. Each group comprised 10 monogamic pairs of each species, which received pelleted or extruded commercial feed. Both diets were formulated to provide for all known nutritional requirements of mice and rats. For Wistar rats, significant statistical differences regarding reproductive performance were limited to the 1st-2nd and 5th-6th parturition intervals (P < 0.05). The average parturition interval and number of offspring born were statistically significant in knockout and inbred mice strains fed extruded diet (P < 0.05). Average weight of weaning mice and rats was measured weekly. Animals fed extruded diet presented better weight gain in all occasions (P < 0.05). Food conversion efficiency was greater in inbred mice strains fed extruded diet. Ammonia concentrations, as measured in the mouse cages, were consistently lower for animals fed extruded diet, probably because of better absorption of nutrients and lower feces production. From the results it can be concluded that extruded diet led to a better reproductive performance and weanling weight gain in mice and rats, possibly due to a better uptake of nutrients.

P163 Role of Diabetes Type 1 in Granuloma Formation by Schistosoma mansoni Infection in NOD (Non-Obese Diabetic) Mice

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The role of autoimmune diabetes in the development of infection by Schistosoma mansoni concerning granuloma formation was evaluated in NOD mice. SPF NOD mice from CEMIB/UNICAMP were infected with 10 cercariae of Schistosoma mansoni BH strain and kept 7 weeks in positive pressure isolators at the Parasitology Department. The diabetes test Self-Stik resulting color changes using urine samples was performed and the last
lecture was determinant for the establishment of the groups: no diabetic, diabetic male and female, diabetic pregnant. Hema-
toxylin and eosin-stained of liver sections and granuloma measurements were determined using a computer system to take into account two perpendicular diameters crossing the cen-
ter of egg. Statistical analysis were performed to granuloma ellipse area using Tukey’s test. Our results showed that the size of granu-
loma is related with glicosuria level. Animals at the beginning of diabetes and normoglycaemic have the same pattern to granu-
loma formation. Pregnant diabetic showed granuloma formation compared as control no diabetic. The response Th1 during ac-
celerated and destructive diabetes and the shift to Th2 profile in schistosomiasis result on Th1/Th2 dichotomy which promote a mechanism of regulation that inhibit or eliminate the granu-
loma formation in diabetic animals.

P164 Schistosoma mansoni Infection Results in Increased Survival of the NOD (Non-Obese Diabetic) Mice

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Although of diabetes and S. mansoni is a possible event in sev-
eral geographic areas of the world, very little is known about the possible interactions between these two phenomena. S. mansoni infection induces T helper cell type 2 (Th2)-type cytokines in the liver of humans and mice. Recent reports have been shown the evidence that Th1 responses are detrimental and Th2 responses are protective against diabetes type 1. In this study we took advan-
tage in NOD mice which has a Th1 profile and has a limited survival after the expression of the diabetes. The animals were maintained in plastic isolators and the survival was monitored. The results were compared with those obtained in a diabetic non-infected control group. Diabetic infected animals had increased time survival and no mortality was registered in infected diabetic pregnant group. In specific-pathogen free conditions, the untreated diabetic mice survive for 3-4 weeks after the first detection of glycosuria. Diabe-
tes was assessed by measurements of urinary glucose levels. The mortality was monitored until the acute phase of the infection by the parasite. The animals were periodically evaluated concerning sanitary conditions to certify the health of SPF animals. The in-
crease of survival in pregnant diabetic females has been seen in 100% of the cases. In this case, no mortality was associated with the humoral immunity, a Th2 phenomena in pregnancy, the in-
creases in insulin secretory response and B-cell growth associated with the development of type-2 responses during schistosomiasis. Our data reveal that the enhancement of survival in infected NOD groups suggests temporal changes in normal progression of di-
betes. Similar events may control the outcome of immune responses in human S. mansoni infection.

P165 Canine Parvovirus: Use of Monoclonal Antibodies for Di-
agnostic and Therapeutic Purposes

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Two monoclonal antibodies were generated against both Vp1 and Vp2 capsid proteins of canine parvovirus (CPV-2), using polyethilenglicol (PEG) fusion between Balb/c mice lymphocytes and Sp2 myeloma. For diagnostic, the Vp1 antibody (IgG1) was used in the solid fase (polyvinyl chloride) in order to capture the virus primary in only a drop of fecal material and/or intestinal smears. Then, the Vp2 antibody (IgG2b) was conjugated with peroxidase enzyme as second antibody in a direct ELISA (Enzyme Linked Immunosorbent Assay) test. The method provided an easy and specific analysis in the field in only 20 min., without special materials and equipments. The efficiency of the test was 97.6% and sensibility 100%. For therapeutic purpose, the Vp1 monoclonal antibody was used at 100 µg/kg doses in infected dogs with CPV-1, with two applications in 24h; this treatment had been used in more than 400 dogs with a success of 98.2%. We describe the methods for monoclonal antibodies generation and their use in CPV diagnostic and treatment in dogs of differents strains. These results have proved a very good sanitary control of the desease in labora-
dory dogs colonies, where the incidence could be important.

P166 Prevalence of Antibodies against Bartonella henselae in Fe-
line and Human Population with Occupational Risk in Tree Regions of Chile

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The prevalence of antibodies against B. henselae (Bh), in the gen-
eral population and in its natural reservoir, the Felis domesticus, is unknown in Chile. Thus, the following was established: determine its prevalence in the human population with labor risk (veterin-
arians, assistants, etc.) and in cats coming from regions VI, X and MR, and isolate and identify the bacterium and correlate the ser-
sum positivity of the animals with the geographic region, presence of factors and vectors that predispose to the illness. Antibodies IgG specific to B. henselae were determined in 187 serums of cats of different ages, and sanitary and dwelling conditions, coming from three regions in the county, as well as in serums of 107 persons with occupational risk. The IF indirect technique was used (in di-
lution 1/64), with antigens supplied by the Center for Disease Control (CDC, USA). Sixty samples were subjected to haemocultures using lysis centrifugation, sowing in lamb blood and chocolate agar at 37°C, CO2–10% for 6 weeks. The colonies identified by morphology and dye that corresponded to Bh were re-sowed and confirmed by PCR in the CDC. The general preva-
lence of feline B. henselae was 85.6%; by city: 96% in Santiago, 74.6% in Valdivia and 79.2% in Coquimbo. The stray cats showed signifi-
cant differences in the proportion of infected in relation to the domestic cats. The presence of fleas and the age of animals did not have a significant influence. Only 41.7% hemocultures were positive (25/60), and in 11 of them B. henselae was retrieved and confirmed by PCR. Of the 107 persons studied, 92% declared that they had had accidents with animals, however only 10.5% were serumpositives. It was concluded that B. henselae is an endemic infection in the feline population of three regions of Chile. The quality of life of cats has a significant influence in the proportion of infected, not so the presence of fleas and age. The prevalence of specific antibodies between the persons with labor risk is propor-
tionately low, in spite of the high frequency of exposure to scratches and bites.

P167 Planning an Isolated Area in the UE #3

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At the beginning of microbiological research, the use of patho-
genic microorganism and manipulation with high-risk agents have obligated to the scientist to work in stricter safety conditions each
time in order to assure personnel and environment protection of personnel and animals. In our Laboratory Animals Production and Experimental Unit we are planning to work with microorganisms that could cause health problems to the personnel involved. For that reason we have planned an isolated area following all the lineaments of the National Institute of Health of the United States in order to reduce any professional risk. We took advantage of an experimental unit area already existent that was rebuilt, equipped and conditioned in agreement with all requirements pointed out by diverse specialized institutions. Furthermore, we designed the technical methods that should be follow strictly to ensure a biological seal at all times; the quality should be guaranteed through periodic monitoring and validation in all the existent systems (air flow, filters, air flow workbench, type II Class B, autoclave, etc.), defining a preventive maintenance calendar that allow us to maintain the correct function of all the equipment. The correct and specialized training is another goal in this new project.

P168 Ergonomics Applied in Captive Nonhuman Primates in Mexico City: Preliminary Report

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Laboratory animal science ergonomics is relatively new, only have the biohazard and preventive medicine assessment, the which enrich this discipline. The protocol using was a sensorial assessment along of video, photography and flow diagrams; were taken somatometric measurement a 28 individuals of four centers of research with different kings works, as soon as 53 monkey rhesus of research center CAMINA A.C. The analysis of results show than the nonhuman primate buildings are inadequate because the spaces do not meet the measurements required for both humans and nonhuman primates. Before buildings spaces for nonhuman primates, it is important to analyze the ergonomics of both human and nonhuman primate populations, considering the measurement extremes (the smallest and the biggest) to avoid inadequate design of buildings.

P169 Medical and Health Assessment of Captive Nonhuman Primates in Mexico City

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Objective: A clinical and health assessment based on the requirements or recommendations prescribed by national and international authorities on captive nonhumans primates was performed on 55 rhesus monkeys (Macaca mulatta) and 44 green monkeys (Cercopithecus aethiops). The protocol included a complete physical examination, haematologic and biochemical analysis, a serology analysis (herpes virus B, hepatitis virus, tuberculosis), parasites and bacterial test (Salmonella spp, Shigella spp) in fresh fecal sample, X rays and ultrasonografies analysis, and a bromatologic analysis at the food was also make.

The result showed a hipoproteineim light and severe hipoproteineim in macaques and vervets respectively the herpes virus was positive in 80% of the colony, in the serologic analysis, Balantidium coli only was found in macaques, Balantidium coli, Capillaria spp, Trichuris trichiura and Entamoeba spp were found in Cercopithecus aethiops, and another hand the bacteriological assays was negative. With the result we suggested as treatment performance desparasitiation, diet complementary and environmental enrichment in both species macaques and green monkeys. Mexico is a place of transit of different species of nonhuman primates to several countries, its shows a potential dangerous by their management and our study remarks the importance to realize this periodic assessments to prevent diseases that have epizootic potential, as well to have animals healthiest physical and physiologically.

P170 Topics of Environmental Enrichment in Captive Nonhuman Primates in Mexico

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The environmental enrichment is necessary in all research line that available nonhuman primates animals because their necessity ethologics are bigger. The topics of enrichment performed in 55 nonhuman primates (Macaca mulatta) in captivity were social, cognitive, feed, sensorial and physical, with the purpose to avoid abnormal behaviors. An ergonomic equipment as instrument to evaluate the colony was used innovator food. The evaluations were make two hours each morning and afternoon by 1 month. A reduced of aberrant behaviors with arise affiliatives and social behaviors as also the animals had more alerts and actives conduct. The performance of diversity environmental enrichment improvement the social behavior and provide psychological well-being nonhuman primates in captivity.

P171 Influence of a New Design of the SPF Colonies in Breeding and Experimental Laboratory Animals Units on Productivity Performance

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The Laboratory Animal Facility of the Faculty of Pharmaceutical Sciences and the Chemistry Institute of the University of São Paulo, re-furbishing the existing facility and implant a project to integrated the breeding and the experimentation areas in accordance with the international standards to assure the quality of the animals. The new areas were provided with:

- Strictly sanitary barriers
- Modernizing and increment of the areas for the animal accommodation with environmental control of all variables.
- Experimental areas with controlled flux of people, establishment of an operational flow to animals and materials to avoid the cross contamination.
- Biosafety areas maintained by air conditioning that create pressure differentials and by the using of HEPA filters in the exhaustion of the air.
The breeding strains were imported from Taconic-USA and introduced in protected areas and maintained under barriers. The productivity performance of the breeding strains was analyzed until 8 months after the new physical arrangements, and after establishing the procedures and routines to minimizing contamination of the animals with unwanted organisms. The productive parameters analyzed were: fertility index (%), productivity index (puppies/female/month) and the parturition interval (days), the data showed respectively: RATS: SD/FCFIQ 85.6%, 10.6, 29.2; WH/FCFIQ 81.0%, 9.7, 30.8; F344/FCFIQ 64.8, 6.5, 39.3; NIH-nuddFFCQIQ 88.8%, 5.4, 38.0; MICE: SW/FCFIQ 77.8%, 11.1, 30.8; C57BL/6FCIQ 66.7%, 4.7, 37.3; BALB/cFCFIQ 77.0%, 5.3, 38.5; BALB/c-nuffDFN9FCFIQ 40.0%, 2.0, 23.3. This indices are using like indicators which are suitable for further planning purposes.

P172 Detection of Estrus through the Presence of Crystal Patterns in Female CD1 Mice Using Saliva Smears

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The objective of this study was to detect the prooestrus and estrus phases in CD1 female mice by examining mineral arboreal crystal patterns in saliva smears. The control was a vaginal smear with hematoxylin and eosin stains. The arboreal forms are crystals of sodium and calcium chloride normally present in female mucous secretions during the prooestrus and estrus phases. Twenty female CD1 mice, 10-12 weeks of age, received oral and vaginal washings for fresh saliva and vaginal smears, respectively, for five consecutive days during a period of four weeks. The smear was observed at 40× magnification for cellular differentiation and the formation of arboreal crystals. Results were similar for the crystal formation technique in 82% of the mice in the estrus phase and in 95% of the mice in the proestrus phase compared with the vaginal smear, and were evaluated using average comparison methods. The technique was not able to differentiate between the metaestrus, diestrus and anestrus phases, because crystals form in the other phases. The differentiation between the proestrus and estrus phase is made by the shape and thickness of the arboreal forms. The arboreal crystal technique is useful to detect the prooestrus and estrus phases and to obtain females for programmed gestations; it reduces the possibility of injuries and/or infections caused by handling of the females in the vaginal smear technique, is easily done and processed, can be read without difficulties, and is less expensive than the hematoxylin and eosin stain technique.

P173 Parameters of Clinical Chemistry in B6D2F1/J Mice (Mus musculus)

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Little information about the chemical parameters in some strains of mice is available, but with the many strains of mice it is necessary for researchers to be able to compare these parameters with those of other strains in order to make informed decisions about the animal model that they choose for their research project. The purpose of this study is to provide more information about ten chemistry elements in blood and urine. For this purpose, 30 mice (females and males of different ages) were housed in polycarbonate cages and were fed PMI 5001. Blood and urine samples were collected from each mouse, and all the samples were analyzed in a Bayer RA-100 machine. Normal values in mice were compared for sex and age.

P174 El Cerdo como Modelo Biológico para Evaluar el Efecto de la Fortificación De La Tortilla de Maíz con Soya y Vitaminas

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Con el objeto de evaluar el efecto de la fortificación de la tortilla sobre la condición corporal y valores hemáticos se uso el cerdo como modelo biológico. Se llevó a cabo un experimento con un diseño aleatorio, utilizando 18 cerdos machos de 45 días de edad distribuidos en 6 grupos, con tres repeticiones. Los cerdos fueron alimentados durante 22 semanas con los siguientes tratamientos: Tortilla de nixtamal (TN), Tortilla de nixtamal y soya al 4% (TNS), Tortilla de nixtamal con vitaminas y minerales (TNV), Tortilla de harina de nixtamal (TH), Tortilla de harina con soya al 4% (THS) y Tortilla de harina con vitaminas y minerales (THV). Los cerdos fueron alojados individualmente en corrales de 4 metros, el alimento y el agua de bebida se dio ad libitum, el alimento fue pesado diariamente y los cerdos cada semana. Se obtuvieron muestras sanguíneas cada 15 días, los cerdos fueron sacrificados de acuerdo a la norma oficial mexicana NOM-033-ZOO 1996 al sacrificar se evaluó la proporción de músculo, hueso y grasa, densidad ósea, las variables evaluadas fueron: ganancia de peso, conversión alimenticia, y los hemogramas. Resultados. En los cerdos alimentados con THS y TNS la ganancia de peso, conversión alimenticia y densidad ósea presento diferencias significativas (P < 0.05). Por otra parte, la conversión de músculo en TNS seguida de THS fue mayor (P < 0.05). Con la fortificación con soya y vitaminas los valores de hemoglobina y hematócrito fueron mayores para THS y TNS. (P < 0.05). Con los resultados anteriores se sugiere que la fortificación de las tortillas con pasta de soya se puede mejorar el desarrollo corporal y condición general.

P175 Match-Up System for Outbred Strains in Small Reproductive Colonies

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The pattern of mating in small animal facilities, which use outbred strains, had not been well documented. In our case, it was necessary to establish a balance in the mating system to avoid the overproduction of animals and minimize the cost of resources. In order to develop the exogamic crossing of a stock of CD-1 mice, a minimum of 14 pairs were settled down (foundation stock) and 14 groups were matched up 3:1 in three different lots (extension stock). The match-up breeding method was used to avoid the maximum homozygocity (for 10 of 25 pairs). The best results in relation to the demand for teaching and investigation were obtained by subdividing the mating of the extension stock in two parts, with one week’s difference between each. Implementation of this system aided a modification in the breeding system that allowed avoidance of the maximum consanguinity to maintain necessary animal production and diminish over-production in animal facilities with limited economic recourses.